DEVELOPMENT AND EVALUATION OF METHODOLOGY FOR THE CHARACTERISATION OF EFFLUENT DISCHARGES

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ABSTRACT

The establishment of a meaningful framework within which to apply management decisions concerning the present and any future water quality standards requires that data on the composition of effluents and their impact on receiving waters be available. The collection of these data requires the application of methodology which will allow for an adequate characterisation of the effluent discharge. Previous work, and the results of a questionnaire sent to Regional Water Boards, indicated an acute need for methodological guidelines to be established with respect to the sampling and analysis of effluent discharges. This study is concerned with the development and evaluation of methodology for the characterisation of: (i) a municipal sewage effluent, (ii) a freezing works effluent, and (iii) a dairy shed effluent, in terms of the currently used criteria of water quality, and phosphorus and nitrogen forms.

To determine the best preservative treatment for nitrogen and phosphorus forms in filtered and unfiltered samples of sewage and freezing works effluents, two preservative amendments (mercuric chloride and N-Serve) plus one control (unamended) were tested in combination with three storage temperatures (room, 4C, -10C) over a 30-day storage period. No one preservative treatment was ideal for all N and P forms studied for both effluents. In general, however, it was concluded that:

(i) -10C was the best storage temperature for dissolved inorganic phosphorus and dissolved ammonium nitrogen but was unsuitable for analyses involving particulates (total ammonium nitrogen, total Kjeldahl nitrogen) or organics (dissolved Kjeldahl nitrogen, total Kjeldahl nitrogen) for which refrigeration at 4C was the best storage temperature, and

(ii) No preservative amendment was necessary for sewage effluent samples provided samples were stored at 4C

or -10C (as appropriate); however, even with temperature control the addition of 50 mg $HgCl_2$ ℓ^{-1} of effluent greatly assisted in the preservation of N and P forms in freezing works effluent.

The survival of indicator bacteria (total coliforms, faecal coliforms, and faecal streptococci) in samples of each of the three effluents was investigated. Membrane filtrations commenced within 30 min. of sample collection and after 3, 6, 9, 12 and 24 hours storage at room temperature and 4C. The results indicated that samples of the effluents under consideration could be stored for up to 6-9 hours at 4C before appreciable changes in their indicator bacterial composition became apparent.

Monitoring studies were carried out on each of the effluent discharges. Sampling was from 1 to 3 days duration at intervals of not greater than 2 hours. Each effluent was characterised in terms of N and P forms, indicator bacteria, oxygen-demand parameters, solids, and pH. In addition UV absorbance at 250 nm and absorbance at the dominant 'visible' absorbance peak was determined on filtered samples. Correlation coefficients of up to 0.98 between absorbance at 250 nm and a variety of organic or organically-related parameters indicated that UV absorbance at this wavelength could be a monitoring aid for effluent surveillance programmes.

Time- and flow-based variations in flow, and concentration and loading of analytical parameters in the effluent discharges were discussed in terms of possible factors affecting the determination of sampling frequency. A computer integration method and probability plots were used to determine the sampling frequency needed to characterise concentration and loading of analytical parameters in effluents to within prescribed limits of accuracy.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES*

*Due to space considerations, appendices for effluent monitoring runs are not included. These data are stored on a computer tape file and are available on request from the Department of Soil Science.

CHAPTER 1 INTRODUCTION

Concern about the deterioration in the quality of natural waters in New Zealand has received increasing attention in recent years. Population, agricultural, and industrial growth has resulted in increasing discharges of a range of effluents to waters and there is considerable evidence accumulating to suggest that water quality is declining in many areas (Fish, 1971; Syers, 1974; Timperley, 1975).

The law regarding water pollution in New Zealand is principally outlined in a 1971 amendment (Water and Soil Conservation Amendment Act (No.2) 1971) to the Water and Soil Conversation Act 1967. An integral part of the amendment was that all waters in New Zealand should be classified according to actual or intended use. Responsibility for classification was given to each Regional Water Board (RWB) (constituted in the 1967 Act), subject to the approval of the Water Resources Council (constituted in the 1971 Amendment Act). The issue of all rights to discharge waste also became the responsibility of the RWBs.

Since the enactment of this amendment, the Act has come in for a great deal of criticism from scientific, legal, and administrative circles. One of the main criticisms has been levelled at the limited number of parameters in the water classification classes, the meaningfulness of these parameters, and the actual values of the parameters that are used (Walker, 1975).

Weaknesses exist in the definition of standards. The only parameters which can objectively be monitored are pH, temperature, dissolved oxygen (DO), and total and faecal coliforms. Toxic substances are judged illegal if 'dangerous for consumption by humans or farm animals' or if their concentration causes 'destruction of natural aquatic life'. Colour and clarity of natural waters 'shall not be changed to a conspicuous extent'. Discharges that are not 'substantially free' from suspended solids, grease, and oil, are not permitted to enter waters. Such subjective terms are virtually

unenforceable. A schedule of allowable concentrations of these substances would have been beneficial from an enforcement point of view (Walker, 1975), though United States experience has shown that many difficulties could arise by the premature implementation of such a schedule (Sliter, 1974).

Another criticism which may be levelled at the Act is its lack of procedural definition with respect to the taking of samples. In the case, for example, of DO the Act does not specify when a sample is to be taken and what account should be taken of flow conditions. The number of samples is specified for coliform standards, but this too is a source of contention. Data for an infringement is based on the median of not fewer than 5 samples taken over not more than a 30-day period. However, coliform bacteria are known to persist in natural waters for varying lengths of time under different environmental conditions (see Chapter 5) and there could be wide differences in the median levels obtained, depending on when the sampling was carried out.

It is the values put on the bacteriological standards which have generated the most discussion of any of the standards in the Act. These standards were based on the 1968 edition of *Water Quality Criteria*, as a member of the committee responsible for recommending the standards candidly admits (Carrie, 1973). Faecal coliform standards for Class C waters (recreational and bathing) are more strict ($\leq$ 200 faecal coliforms per 100 ml) than are those for Class B waters (may be used for a public water supply with treatment). Recent work has shown that public health risk in bathing is not caused by ingestion of faecal material but rather by ear, eye, and throat infections which are related to bathing density rather than faecal pollution (Wolf, 1972). This appears to have at least been recognised by the committee recommending coliform standards as Carrie (1973) commented that the faecal coliform standard for Class C waters was only included because no other parameter could be thought of by which the degree of sewage pollution could be measured.

The actual values of coliform standards are largely immaterial, however, as any values (unless large error limits were included) would virtually be unenforceable. This is due to the lack of definition of the bacteria or methods and media for their enumeration. Carrie (1973) claimed that this lack of definition gave rise to a very large and very desirable tolerance which would have to be accounted

for before prosecution for an infringement could succeed. While most microbiologists would agree that the lack of definition will give rise to a large tolerance, considerably fewer would agree that it is desirable.

There are no nutrient standards in the Act as it now stands. In the 1971 amendment a blanket classification, designated Class X, was put in the schedule. This class could be superimposed over any class that was in danger of becoming eutrophic. The wording of the defining clause; 'Discharges into the waters shall have not less than 80% of the total phosphate content as phosphorus, ... , removed by such method of treatment as the Water Resources Council approves', was clearly nonsensical and the clause was later removed. It is now included in the body of the law as 'by adding the symbol X to the classification the Council may indicate that the area of water in respect of which the symbol is added is sensitive to enrichment'. What measures are then taken is not mentioned. The lack of nutrient-oriented receiving water standards is to be regretted. As Walker (1975) pointed out, there is belief among some sectors of the scientific community that nutrient levels are more significant in the New Zealand situation than the usual organic or physical criteria.

Although maintenance of water quality in New Zealand is enforced by the application of receiving water standards, these can only be achieved by applying effluent limitations to waste discharges. In other words, receiving water standards and effluent standards are not alternatives, they are complementary to each other (Gunn and O'Grady, 1972). The imposition of the effluent standards necessary to maintain receiving water quality is the responsibility of the RWBs when issuing the discharge rights.

Some confusion as to the manner of application of standards has existed, as some Boards have granted rights to discharge waste subject to conditions which simply require the discharger to maintain certain receiving water standards taken directly from the schedule to the Water and Soil Conservation Amendment Act (No.2) 1971. In reviewing the directive issued by the National Water and Soil Conservation Organisation (NWASCO) to RWBs, Walker (1975) observed that it was the intention of the former organisation that the conditions on rights are to be defined in terms of effluent standards. In this manner the responsibilities of the discharger are clear and

the responsibility of the RWB to maintain receiving water quality is not abrogated. Furthermore, the effluents are to be related to the rivers assimilative capacity within the limits of the defined receiving water standards.

In the same thesis, Walker (1975) researched the attitudes and technical awareness of the RWBs with respect to their rôle in water quality surveillance. His findings may be summarised as follows:

(i) NWASCO has been slow in recognising the need for water quality data collection at a regional level. As a result of insufficient financing, Boards still have inappropriately trained staff.

(ii) Despite several in-service training courses covering sampling, analytical and preservation techniques, many Board staff had over-confident and simplistic views of quality and testing.

(iii) In-service courses on sampling techniques have tended only to repeat principles of simplified methods or have referred to 'obsolete' technical papers, and have not provided Boards with operational techniques.

(iv) Technical advisers from NWASCO appeared to favour wet chemical methods despite overwhelming overseas experience with, and support for instrumentation within New Zealand.

(v) Some technical services being offered by NWASCO are beyond the general level of technical experience and understanding of Board staff. It was felt that offering such services as computer simulation for DO prediction was ahead of its time.

(vi) The concept of monitoring programmes to establish quality conditions and to characterise the effects of wastes was not understood in a number of cases.

(vii) Only Boards with major inland industrial and municipal discharges saw their rôle in quality control as surveying to establish allowable waste loads.

(viii) There was little attention to accounting for possible variation of waste load. Only one Board considered that understanding the character of the waste, both in quality and quantity, was important.

Thus, although statements have been made at national policy level as to what RWBs should do in applying the standards set out in the Water and Soil Conservation Amendment Act (No.2) 1971, few technical guidelines have been given on how to apply this policy.

This study presents:

(i) A more detailed survey of the methods used by RWBs to monitor effluent discharges.

(ii) An intensive study of three effluent discharges in the Manawatu region with particular emphasis on the sampling, preservation and analytical techniques necessary for their adequate characterisation.

CHAPTER 2 MONITORING OF EFFLUENT DISCHARGES IN NEW ZEALAND — A SURVEY OF REGIONAL WATER BOARDS

2.1 Introduction

In order to assess;

(i) the types of major effluent discharges under the jurisdiction of RWBs, and

(ii) the technical resources and knowledge available to monitor these effluent discharges,

a questionnaire (Appendix 1) was sent to all RWBs (including Commissions and Authorities) in the country. As the main purpose of the survey was to obtain an 'overview' of the methods used in New Zealand to monitor discharges and not to highlight the deficiencies or attributes of any one Board, an assurance of confidentiality was given.

Written replies were received from 14 of the 20 Boards. Of these, 3 Boards declined to answer the questionnaire but some relevant information was obtained from them.

2.2 Effluent discharge types

The major industrial effluent discharges in New Zealand are almost entirely associated with agricultural and horticultural processing industries (Table 2-1). Municipal sewage was the most frequently cited major effluent discharge. Two Boards commented that they had only limited authority over major municipal sewage discharges in their region, as these discharges were covered by separate empowering acts (Drainage Board Acts).

Table 2-1 Summary of major effluent discharges administered by Regional Water Boards

Board	Effluent source																
	Sewage (City)	Sewage (Borough or smaller)	Fertiliser works	Pulp &/or paper mill	Freezing works	Dairy factory	Wool scouring	Fish processing	Farm	Truck wash	Fruit and vegetable processing	Metal extraction	Brewing	Fell-mongery	Aluminium smelting	Wine manufacture
A		8	2													
B	1	6		1	1	1										
C	2			3	1	3	1									
D	1	4			1			1	1	1						1
E	2	2	1	1	3						2					
F	1	4			2		1			1		1				
G	1	3			2	3							1			
H		4			1				2							
I		3			1	2		1	2		1					
J	1	5			3									1		
K	1		1	1	4	1									1	

2.3 Sampling of effluents

Four Boards indicated that they did not sample effluents at all. The same number of Boards unreservedly replied that they routinely monitored their effluent discharges. Two others only sampled if it was suspected that the discharger was violating his right, and the remainder indicated a haphazard approach to effluent sampling.

The frequency with which a sampling exercise was undertaken for any particular discharge covered almost the whole spectrum, from weekly to yearly. Two Boards indicated that they could not define the frequency but that it was irregular. The number of samples taken on any particular sampling exercise showed similar variation though the most popular was 2-3 per day. Three Boards took 7-12 samples per day and one indicated that 13-24 samples were taken per day. Board staff appeared to appreciate the relationship between the number of samples and the ability to meaningfully analyse them, as the number of samples taken appeared to be roughly related to technical resources, as indicated by later answers.

Grab sampling is the method used by all RWBs, and only one Board had any automatic sampling equipment. This was a peristaltic pump type of its own manufacture and was used for a specific project rather than effluent monitoring. Several other Boards stated that they would very much like automatic sampling equipment but that cost precluded its purchase. Flow-weighted composite sampling was used for one isolated discharge.

Only 2 Boards routinely sampled effluents outside of normal working hours though 2 others indicated that a start in this direction was anticipated.

2.4 Assessment of loading

Of the 11 Boards who answered the questionnaire, 5 had not made any assessment of loading for any parameter from any of their effluent sources. Of the remaining 6 Boards, approximately 50% of their 10 major discharges had been assessed. One cannot but wonder how the Boards enforce discharge rights for effluents in which loading has not been measured, or even how they arrive at values for discharge in the first place, as rights are generally issued on a $kg.day^{-1}$ basis. One Board which didn't answer the questionnaire replied that all rights to discharge were written 'in terms of instantaneous units ($mg.\ell^{-1}$) rather than in maximum daily quantities in order to avoid the need for 24-hr sampling'.

In order to make an assessment of loading, flow data are required. Ten Boards answered the question on flow meter installation; covering a total of 100 discharges. On a regional basis, permanent flow metering devices were installed at between 0 and 80% of the total major effluent discharges. Nationally, only 40 of the 100 major discharges had permanent flow meters installed. Of these, 30 were the recording (continuous) type while 10 gave an instantaneous reading only. As, in most cases, there exists a good relationship between flow and load (see Chapter 7), the advantages of having a continuously recording flow meter are obvious. In fact, one Board has made an assessment of annual loading on the basis of a simple flow-weight conversion. This type of conversion may be satisfactory but only after studies of the characteristics of the parameters for which a loading estimate is wanted.

Thirty-four of the 100 major dischargers reported results of self-monitoring to the RWB. The trend appeared to be that any new discharge rights, or old rights which came up for renewal, have such a requirement.

2.5 Analytical capabilities

Most Boards have carried out the analyses required to maintain the receiving water standards of the 1971 Amendment Act, i.e., 5-day biological oxygen demand (BOD_5), dissolved oxygen (DO), pH, suspended solids (SS), temperature, and total and faecal coliforms. One Board only has equipment for DO, pH, and temperature while another has a small laboratory equipped but has no finance to hire someone to run it. Only 2 RWBs have used the chemical oxidation demand (COD) analysis for effluent monitoring, one of which is also the only one to have used faecal streptococci as a bacterial indicator. Most of the other Boards have done both total and faecal coliform determinations on effluents though several have done just one or the other.

Most RWBs contract out nutrient and toxic substance analyses. Analyses which are done by the RWB or contracted out vary from Board to Board. One RWB has facilities to do all analyses specified in the questionnaire, whereas others contract out all analyses except DO, pH, and temperature. One Board appeared to have been forced to develop a reasonably good analytical set-up by its isolation from suitable contracting facilities.

Only one RWB indicated that nutrient standards had been included in any discharge right. One of these standards is currently under appeal by the company concerned. Despite the fact that the other Boards have no enforcement interest with regard to nutrients (N and P), most of them appear to show some scientific appreciation of the importance of N and P in the aquatic environment. This is reflected by the 60% (of the 11 answering the question) who have made some kind of P determination (DIP, TP) and 70% who have made N determinations (NH_4-N, NO_3-N, or TKN).

There does not appear to be a similar degree of interest in heavy metals or other toxic substances. Only 30% of the Boards replying showed any interest in this area and this was related to standards specified on discharge rights. These related to effluents from fertiliser works (fluorides and sulphates), fellmongeries and freezing works with fellmongery operations (sulphides), aluminium smelters (Cd and Hg), and woodpulping plants (phenols).

2.6 Preservation of effluent samples

It was evident that, in general, Boards showed little appreciation of this important area. Most cited the manganese sulphate-alkaline iodide method of fixing DO on the site. Only 50% (of the 10 RWBs answering this section) used refrigerated storage for delays in BOD_5 analysis whilst 2 used freezing, a practice which is not recommended (Benedek and Najak, 1975). Only 4 Boards saw a need for immediate refrigeration of samples prior to bacteriological analysis. One Board used freezing; a method totally inadequate for bacteriological analysis due to lysis of cells. Another Board indicated that virtually all analyses were done immediately! While being a commendable practice it is doubtful whether this can ever be so in reality due to travel time from the effluent source to the laboratory.

Two RWBs used preservative amendments for parameters other than DO. One used $HgCl_2$ as a preservative for all nutrient analyses, while another used this same preservative for NH_4-N only. The same Board also used zinc acetate as a preservative for sulphide analyses. Three other Boards used either refrigeration or freezing as a preservative technique for N and P forms.

2.7 Future needs

The general comments offered by Board officers indicated a wide disparity in resources and attitudes. Only one Board appeared to appreciate fully the problems (logistical and technical) of adequately monitoring effluents. This Board's Water Resources Engineer was adopting a method developed by the U.S. Environmental Protection Agency (1975) which is, in effect, a cost-benefit analysis of the potential damage which the effluent could cause to a receiving water and a rationalisation of the resources available to monitor it. This type of approach is to be highly recommended and there appears to be a need for a similar New Zealand publication specifically for RWB use.

Although several Boards showed complete indifference, most Boards were acutely aware of, and interested in the problem of effluent monitoring. One Board commented that it has just equipped its water quality laboratory with funds made available by Government, and if the questionnaire were to be sent to them in a years time, far more positive answers would result. Another Board is about to commence a survey on pollutional and nutrient parameters in its area, under NWASCO sponsorship. This it was felt, marked the commencement of adequate monitoring in this region.

Other Boards, however, were less optimistic in their outlook and less benevolent to NWASCO in their attitude. One Board, for example, remarked that a recent Board request for funding for a small spectrophotometer and Millipore Bacto equipment had been turned down. This Board had no funds allocated for pollution control and was attempting to cover required tests by utilising existing (untrained) staff to undertake these duties in addition to normal work. A recent survey suggested that the Board required $50,000 plus 2 full-time qualified staff to cover minimal surveys required to meet legal obligations under the Water and Soil Conservation Act. This Board (and several others) commented that one of the main problems lies in the saddling of RWBs with the 1967 Act (and 1971 Amendment) without making any provision for adequate financial sourcing and staffing.

From the replies received to the questionnaire and also from the survey carried out by Walker (1975), it is apparent that there is an acute need for technical programmes to be set up to provide information for Boards on methodology for the sampling, preservation, and

analysis of effluents. There is also a need for characterisation studies on different effluent types. Because of the type of industry in New Zealand (dominantly agricultural and horticultural processing) it is evident that nutrients may be a very important facet of such a characterisation.

CHAPTER 3 MATERIALS AND ANALYTICAL METHODS

3.1 Materials

3.1.1 Sewage effluent

Treated sewage effluent was collected from the outfall of the Palmerston North Sewage Treatment Plant. The plant is a conventional primary treatment plant designed to carry out the following processes:

1. Comminution: The screening and shredding of large solids.
2. Pre-aeration: Removal of grit and heavy solids.
3. Sedimentation: Removal of suspended solids, grease and floating matter.
4. Sludge digestion.

The incoming flow (Figure 3-1) passes through the comminutors which shred the solid substances to a size which minimises pipe and pump blockages and assists the final digestion process. Aeration and grit removal is carried out in the pre-aeration tank. Grit is discharged into a cart and removed from the site. The flow next passes through three rectangular sedimentation tanks operating in parallel each having a total detention time of about 1½ hr. The suspended matter settles on the bottom and sludge collectors consisting of timber scrapers move the sludge to hoppers at one end of each tank from whence it is pumped to the sludge digestors. The final plant effluent is drawn from the far end of each tank by numerous shallow notches set at the water surface. The flow is collected in a channel and after passing through a metering flume is discharged into the Manawatu River. Flow is continuously recorded on a Honeywell recorder.

The digested sludge is disposed of separately in a sludge lagoon.

Sampling was carried out at the outlet of one of the sedimentation tanks (Figure 3-1) after preliminary monitoring (O'Connor, pers. comm.) indicated that the composition of effluent at the outlets of each of the sedimentation tanks or the final collecting channel (inaccessible for routine sampling) was not significantly different.

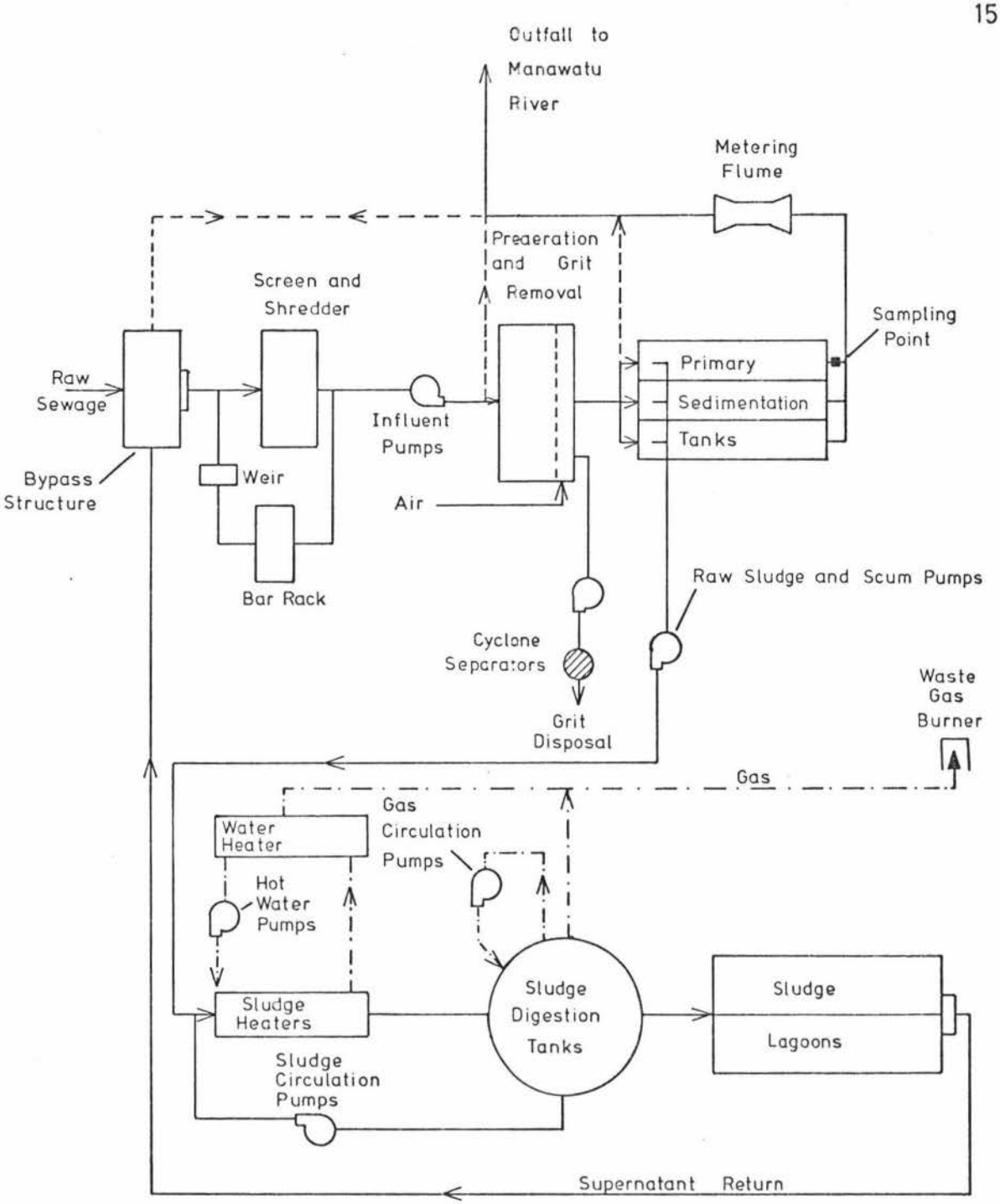


Figure 3 - 1

Flow diagram of the Palmerston North sewage treatment plant.

3.1.2 Dairy shed effluent

Soil filtered dairy shed effluent was collected from the outfall of the effluent disposal area, No.4 dairy unit, Massey University.

The disposal site has been used by the Department of Soil Science to evaluate the effects of land disposal of untreated dairy shed effluent on water quality and pasture condition (Macgregor *et al.*, 1975).

The disposal site consists of 1.6 ha of permanent pasture on Tokomaru silt loam. In order to facilitate drainage of this otherwise poorly draining soil, the area has been tile and mole drained (Figure 3-2). This drainage system is capable of removing in excess of 20 $m^3 . hr^{-1}$ of water (Macgregor *et al.*, 1975).

Untreated effluent is pumped from a collecting sump to a field hydrant by underground piping. Twin sprinklers are attached to the hydrant line and each sprinkler is placed at a 'station' for 24 hours as part of a 10-day circuit. Drainage water from the entire disposal site is collected in a main drain and discharged into a small stream via a V-notch weir, where the flow is continuously recorded with a Stevens F-type recorder.

3.1.3 Freezing works effluent

Freezing works effluent was collected from the outfall of the Longburn Freezing Company's treatment plant, Longburn. The Kiwi Bacon Company, which has a factory adjacent to the freezing works also contributes effluent to the treatment plant.

As with the sewage effluent, treatment is by primary sedimentation. Two primary settling tanks operate in parallel, each with an optimum design loading of 5000 $\ell . min^{-1}$. Floatable material such as fat and accidental inclusions of reject casings, etc., is concentrated by use of sprayers and skimmed off. This material, in conjunction with sludge which is continuously scraped off the bottom of the sedimentation tanks, is pumped to a nearby dump. The final effluent from each tank passes through a flow metering flume and is then piped to the Manawatu River where it is discharged. Flow is registered on an instantaneous flow recorder which also records the cumulative volume of effluent leaving the plant. Tracer studies using dyes has shown it takes approximately 3½ hr for the effluent to travel from the treatment plant to the river (Hinde, pers. comm.).

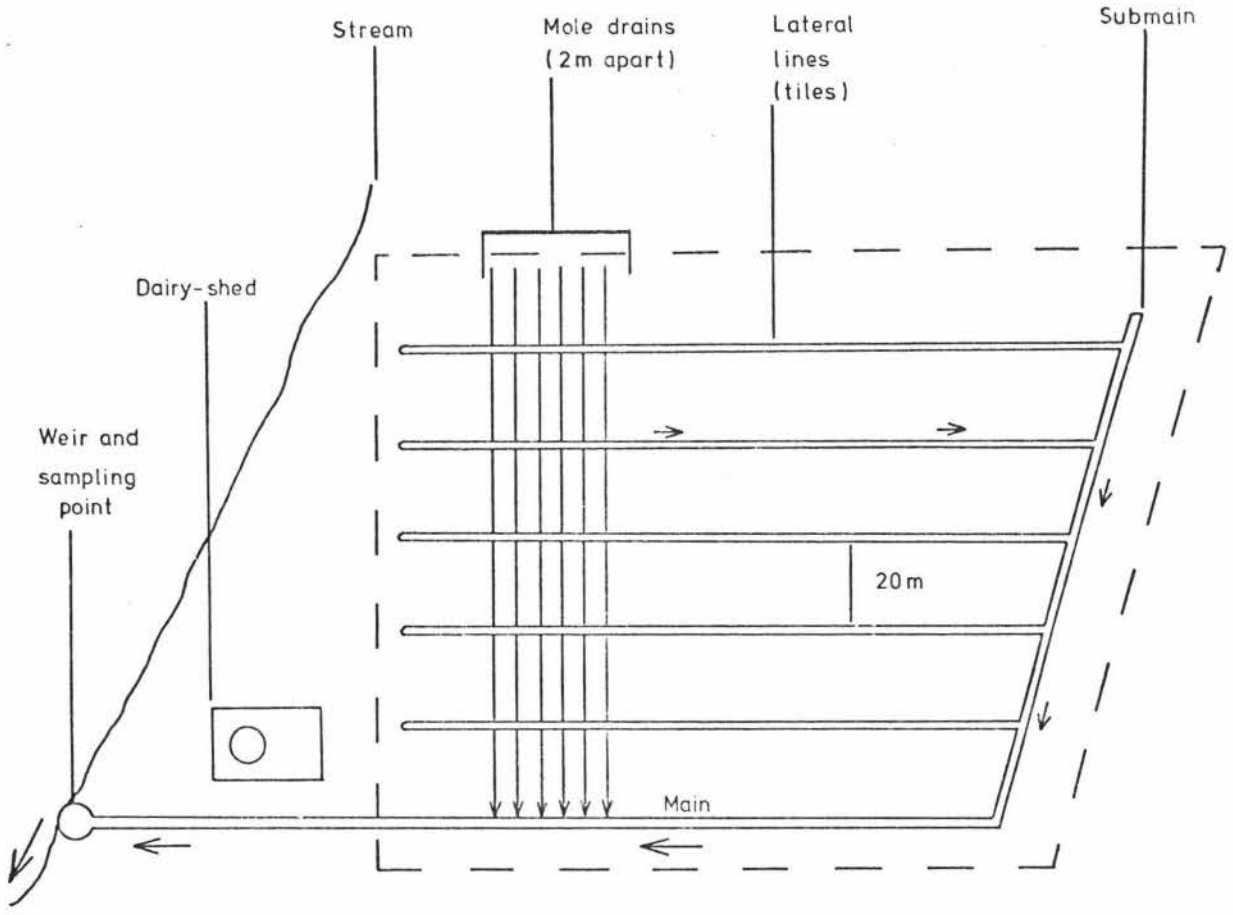


Figure 3-2 Plan of effluent disposal area, No. 4 dairy unit, Massey University.

Sampling took place at the outfall of one of the sedimentation tanks after it was demonstrated (O'Connor, pers. comm.) that the choice of outfall did not bias the results. The outfall to the river was not considered because of its inaccessibility and lack of flow recording.

It should be remembered that most of the treatment of 'potential raw effluent' takes place within the freezing works itself. By-product recovery processes utilise most of the blood, fat, bones, and offal that might otherwise be generated as waste.

3.2 Analytical Methods

3.2.1 Solids

Total solids (TS) concentrations were determined by evaporating weighed samples to dryness overnight at 105 C. Dissolved solids (DS) (only determined on 1 freezing works effluent sampling run) were similarly determined after filtering the sample through 0.45 µm Millipore membrane filters. Suspended solids (SS) (where appropriate) were calculated from the difference between total and dissolved solids.

3.2.2 pH

pH was determined on a stirred sample using a Radiometer PHM61 pH meter.

3.2.3 Indicator bacteria

Total coliform bacteria (TC), faecal coliform bacteria (FC), and faecal streptococci (FS) were enumerated using standard MF procedures (APHA, 1971). DIFCO M-Endo MF broth with incubation for 24 hr at 35 C ± 0.5 C, DIFCO M-FC broth with incubation for 24 hr at 44.5 C ± 0.2 C, and BBL M-Enterococcus agar with incubation for 48 hr at 35 C ± 0.5 C was used for total coliforms, faecal coliforms, and faecal streptococci,respectively. A water bath incubator was used for faecal coliforms due to the precise temperature control needed. However, an air incubator sufficed for the determination of the other two groups.

Preliminary experimentation with conventional Millipore HA membranes and the new Millipore HC membrane showed that the latter membrane gave significantly higher recoveries of total coliforms and faecal streptococci, as well as of faecal coliforms for which they were intended (Green *et al.*, 1975; Sladek *et al.*, 1975). Consequently, Type HC membranes were used for all bacterial groups in characterisation sampling runs in which absolute values were required. Type HA membranes were used for the survival experiments (Chapter 5) in which the maximum recovery was unnecessary, provided a constant recovery was obtained. Economic considerations justified this division.

3.2.4 Oxygen-demand parameters and dissolved oxygen

Five-day Biological Oxygen Demand (BOD_5) was determined manometrically using a Hach BOD apparatus (Hach Chemical Co., 1972). Incubation was carried out at 20 C ± 0.5 C in a temperature-controlled room.

Chemical Oxygen Demand (COD) was determined on a Technicon Autoanalyser using an automated adaptation (Technicon, 1969) of the manual method described in 'Standard Methods' (APHA, 1971). The sample was digested with a potassium dichromate-sulphuric acid mixture in the presence of a silver sulphate catalyst. The depletion of the hexavalent chromium ion due to the oxidation reaction was measured colorimetrically. Potassium acid phthalate ($C_8H_5KO_4$) was used as a standard.

Dissolved oxygen (DO) was measured, *in situ*, using either a Beckman 'Fieldlab' Oxygen Analyser or a Yellow Springs Instrument (YSI) Dissolved Oxygen Meter.

3.2.5 Nitrogen and phosphorus forms

Dissolved inorganic phosphorus (DIP) was determined on a filtered (0.45 µm) sample using the molybdenum-blue reaction (Murphy and Riley, 1962). Absorbance was measured at 712 nm on a Pye-Unicam SP1800 spectrophotometer. Total dissolved phosphorus (TDP, filtered sample) and total phosphorus (TP, unfiltered sample) were similarly determined after acid persulphate (EPA, 1971) and perchloric acid (O'Connor and Syers, 1975) digestion, respectively. Dissolved organic phosphorus (DOP) was calculated as the difference between TDP and DIP, while total particulate phosphorus (PTP) was calculated as the difference between TP and TDP.

Total Kjeldahl nitrogen (TKN) and dissolved (< 0.45 µm) Kjeldahl nitrogen (DKN) were determined on a Technicon Autoanalyser using an indophenol colorimetric measurement following a Kjeldahl-type digestion (Terry, 1966).

Several methods for the determination of inorganic nitrogen constituents were evaluated. The ion-selective electrode (Orion Research, 1975), automated indophenol (Brown, 1973), and distillation methods (Bremner and Keeney, 1965) were tested for determination of ammonium nitrogen (NH_4-N) in effluents, whereas the ion-selective electrode (Langmuir and Jacobson, 1970), automated cadmium reduction (Hendrikson and Selmer-Olsen, 1970), and distillation (Bremner and Keeney, 1965) were evaluated for nitrate nitrogen (NO_3-N) determinations.

Of these methods, the distillation procedure of Bremner and Keeney (1965) was found to be the most suitable for the routine determination

of both NH_4-N and NO_3-N in effluent samples. This assessment is based on the observations that;

(1) both NH_4-N and NO_3-N can be determined on the same sample,

(2) the method is flexible as to the concentration ranges that can conveniently be determined,

(3) the method is least subject to interferences of all the methods evaluated.

The cadmium reduction method for (NO_2 + NO_3)-N is more sensitive than the distillation method and was used to detect small changes in NO_3-N in a sample with time (Chapter 4). The use of the method was not without problems, however, as difficulty was experienced in maintaining maximum efficiency of reduction; a problem which is possibly related to the high dissolved organic component in effluent samples.

The automated indophenol method (Brown, 1973) was rejected for NH_4-N determinations in effluent samples for two reasons. First, the method was too sensitive for the generally high NH_4-N concentrations present in effluents, necessitating either pre-dilution or the introduction of large dilution steps into the method. Secondly, a large positive interference was noted in the determination of NH_4-N in freezing works effluent samples. This interference was investigated but no satisfactory conclusion as to its cause was reached.

While probably the most suitable for *in situ* analysis of effluent streams, ion-selective electrodes were only moderately successful for the analysis of discrete effluent samples. The ammonia electrode was suitable in terms of detection limits and lack of interference, but showed long response times in effluent samples. The low concentrations of NO_3-N (generally < 1 $mg.\ell^{-1}$) in sewage and freezing works effluent rendered the nitrate ion-selective electrode unsuitable.

The distillation method of Bremner and Keeney (1965) was used for the routine monitoring of NH_4-N and NO_3-N in filtered effluent samples.

3.2.6 UV and 'visible' absorbance

Scans for absorbance peaks in effluent samples were done using a Pye-Unicam SP1800 spectrophotometer. Silica (1 cm) cells were used for the visible and UV regions, whereas 4-cm glass cells were used for scans solely in the visible region.

Routine monitoring of UV absorbance (A_{250nm}) was done using 1-cm silica cells. Absorbance in the visible region was measured at wavelengths established from scans obtained using either 1-cm or 4-cm glass cells.

CHAPTER 4 PRESERVATION OF NITROGEN AND PHOSPHORUS FORMS IN EFFLUENT SAMPLES

4.1 Introduction

The preservation of N and P forms in samples of waters and wastewaters constitutes one of the most vexing problems in water quality monitoring and research (Klingaman and Nelson, 1976). Much of the work in the field of preservation of nutrient forms has been done with either river, runoff, or seawater samples. Making comparisons between effluents and waters of such different characteristics is at best a risky practice (Degobbis, 1973). The reason for using a preservation technique on a natural water sample is to bring the sample to biological equilibrium, chemical equilibrium having generally already been reached. Although with effluent samples it is even more important to bring the sample to biological equilibrium, it cannot be assumed that chemical equilibrium has been attained. The relatively short retention time from 'manufacture' of the effluent to its discharge, along with other factors such as variable pH and composition and high suspended solids, make it unlikely that chemical equilibrium has been reached at the time of sampling. Chemical reactions involving precipitation, hydrolysis, or volatilisation of various constituents are likely to be as big a problem in the preservation of effluents as biochemical mechanisms such as mineralisation and nitrification.

Standard Methods (APHA, 1971) stressed the point that: "No single method of preservation is entirely satisfactory, and the preservative should be chosen with due regard to the determinations that are to be made". For these reasons an experiment was designed to test combinations of physical (refrigeration or freezing) and chemical (addition of metabolism suppressents) methods for the preservation of N and P forms in sewage and freezing works effluents.

4.2 Review of literature

Both N (Keeney, 1973) and P (Syers *et al.*, 1973) are subject to a large number of chemical and microbial transformations in the aquatic environment. Thus, unless samples of waters or wastewaters are preserved at the time of sampling, the amounts of N and P forms upon analysis may bear little resemblance to those which were originally present.

Although most of the work on preservation techniques has been done on natural waters, the need for preservation of effluents is usually greater because of their higher biological activity and chemical reactivity (Benedek and Najak, 1975).

Early investigators attempted to use biological preservatives for the preservation of N and P forms in sewage. Formaldehyde (Gage and Adams, 1906) and chloroform (Gage and Adams, 1906; Lederer and Hommon, 1911) were used, largely without success, although it was noted by Gage and Adams (1906) that nitrates could be preserved effectively with 10 to 25 ml of chloroform per gallon. Hellwig (1964) used chloroform to preserve a wide range of parameters used in water-quality monitoring (including N and P forms) in strongly-polluted water samples and reported that chloroform did not stabilise nitrite or nitrate concentrations. Thymol, formalin, KCN and $HgCl_2$ were also tested by this author and only $HgCl_2$ was found to be generally satisfactory.

Acidification with either H_2SO_4 or HCl has been used as a preservative for forms of P (Strickland and Parsons, 1960) and N (Brezonick and Lee, 1966). However, the use of acids is not widely accepted because they promote the conversion of nitrite to nitrate by the Van Slyke reaction (Howe and Holley, 1969; Goulden, 1972).

Mercury chloride has been used extensively as a preservative for N and P forms in both waters and wastewaters. Hellwig (1964) found that 50-80 $mg.\ell^{-1}$ $HgCl_2$ was adequate to preserve nitrite, nitrate, free and aqueous ammonia, and an undisclosed form of phosphorus (presumably DIP) in strongly-polluted surface waters. For raw sewage or trade waste of similar strength, however, Hellwig (1967) used and recommended the higher concentration of 890 $mg.\ell^{-1}$ $HgCl_2$ for effective preservation. At this high concentration of $HgCl_2$, several chemical interferences became apparent. Ammonium and nitrate recoveries were reduced while a strongly positive interference was noted in DIP

determinations. Bubbling H_2S through the $HgCl_2$-amended sample prior to analysis precipitated the mercury and allowed acceptable analyses for ammonium and nitrate, but only aggravated the interference for phosphate. The need for such a high concentration of $HgCl_2$ was questioned by Howe and Holley (1969) who maintained that the complexing and hydrolytic difficulties encountered by Hellwig (1967) might be avoided simply by using a low enough concentration of Hg(II) chloride. They were able to show that 42 mg.ℓ^{-1} $HgCl_2$ in an NH_4^+(aq) spiked distilled water sample did not interfere with the distillation of ammonia whereas 80 mg.ℓ^{-1} $HgCl_2$ caused a 16% deficit in recovery. Further experiments by these authors showed that 80 and 90 mg.ℓ^{-1} $HgCl_2$ did not affect recoveries of NH_4-N from a 4:1, water:sewage mixture.

The use of $HgCl_2$ as a preservative for N and P forms, in conjunction with temperature control, has found favour with investigators of estuarine (Jenkins, 1968), surface runoff, tile drainage, and river waters (Klingaman and Nelson, 1976). These workers found that the addition of 40 mg.ℓ^{-1} $HgCl_2$ and storage at 4 C was a satisfactory technique for the preservation of DIP and soluble inorganic nitrogen forms, although Jenkins (1968) preferred -10 C for long-term storage (30 days) prior to DIP analysis. Goulden (1972) also concluded that the addition of 40 mg.ℓ^{-1} $HgCl_2$, combined with low temperature storage, was the best chemical preservative for common nutrient species in fresh water, but noted that this could not be used for P determinations using the molybdenum-blue reaction due to precipitation. Tillman and Syers (1975), however, showed that the addition of excess chloride or a metabisulphite-thiosulphate reagent eliminated the interference by complexing the Hg^{2+} ions.

Phenyl mercuric acetate (PMA), a mercuric compound which does not complex ammonia during distillation (Keeney, pers. comm.) was found to be a satisfactory preservative for soluble inorganic nitrogen forms in fresh waters (Klingaman and Nelson, 1976), but was unsuitable for DIP. These authors also found that the best overall technique for the preservation of water supplies was unamended storage at -20 C. This finding is in contradiction with an earlier study (Nelson and Römkens, 1972) who found that freezing at -20 C significantly decreased the levels of DIP in unfiltered runoff waters. This, they postulated, may have been due to the exclusion of phosphate ions and soil particles from growing ice crystals, which concentrated the IP and

soil colloids in the liquid phase. This induced an increased phosphate sorption, thus lowering the DIP levels. No significant effect on soluble nitrate or soluble ammonium levels due to freezing was detected by these authors. Freezing has also been found to be a satisfactory method for ammonium preservation in seawater samples (Degobbis, 1973).

An alternative explanation to the effects of freezing on nutrients in water or wastewater samples may be extrapolated from the field of clinical chemistry. When using atomic absorption techniques for trace metal determinations in frozen biological samples (serum, sweat, and urine), Omang and Vellar (1973) observed distinct concentration gradients. Their results showed that these gradients were produced during thawing, when more concentrated solution thawed first and ran to the bottom along the tube walls. No effect was noted in the analysis if the thawed sample was shaken before subsampling. A further explanation is provided by Agardy and Kiado (1966) who postulated that the colloidal character of a wastewater sample will be altered as a result of freezing and thawing processes and that flocculation could occur. Thus chemical species formerly in a colloidal state would no longer form a stable suspension.

From this survey of the literature it would appear that the 'state of-the-art' with respect to preservation of N and P forms in water and especially wastewater samples is far from conclusive. Little work has been done on;

(i) preservation of effluents other than municipal sewage,
(ii) comparisons between the behaviour of nutrient forms of samples stored in a filtered and unfiltered state using different preservative treatments, and
(iii) the use of a nitrification inhibitor for the preservation of inorganic-N forms.

4.3 Materials and methods

Bulk (20-ℓ) samples of effluent were collected from the outfalls to the treatment plants of the Palmerston North Sewage Works and the Longburn Freezing Company Ltd. freezing works. Each bulk effluent was thoroughly mixed before subsampling. Half of each effluent was centrifuged at 13,700 × g for 15 minutes and the supernatants were passed through acid-washed Millipore membrane filters (< 0.45 μm). Bulked filtrates of each effluent were divided into 3 × 2.86 litre subsamples for addition of preservative. The remaining unfiltered portions of each effluent were similarly divided.

Mercuric chloride (14.3 cm^3 of a 10,000 $mg.\ell^{-1}$ $HgCl_2$ solution (50 mg $HgCl_2.\ell^{-1}$ effluent)) was added to one filtered and one unfiltered subsample of each effluent. This concentration is slightly higher than the 40 $mg.\ell^{-1}$ $HgCl_2$ used in water samples (Klingaman and Nelson, 1976; Jenkins, 1968). Similarly 1.452 g of N-Serve (2-chloro-6(trichloromethyl pyridine)) was added to two further subsamples of each effluent (500 mg N-Serve.ℓ^{-1} effluent). This concentration of N-Serve is recommended as being adequate to prevent nitrogenous BOD occurring during the BOD_5 test (Hach Chemical Co., 1972). Two other subsamples of each effluent (filtered and unfiltered) were left without any preservative addition.

After thorough mixing, the subsamples were divided up and stored in 50 cm^3 plastic bottles with plastic caps. One-third of each subsample (19 bottles) was stored at room temperature, one-third refrigerated at 4 C, and the other third frozen at -10 C.

Each effluent was analysed prior to storage and at selected intervals throughout a 30-day storage period. Filtered and unfiltered samples were analysed for the nutrient forms listed in Table 4-1.

Table 4-1 Forms of nitrogen and phosphorus monitored during preservation experiment

Nutrient form	Abbreviation	Reference
Dissolved inorganic phosphorus	DIP	Murphy & Riley (1962)
Dissolved ammonium nitrogen	DNH_4-N	Bremner & Keeney (1965)
Total ammonium nitrogen	TNH_4-N	Bremner & Keeney (1965)
Dissolved nitrate nitrogen	DNO_3-N	Hendrikson & Selmer-Olsen (1970)
Dissolved Kjeldahl nitrogen	DKN	Terry (1966)
Total Kjeldahl nitrogen	TKN	Terry (1966)

During the DIP determination on samples containing $HgCl_2$ as a preservative, an excess of Cl^- ions (as $NaCl_{(aq)}$) was added in order to prevent precipitation during the molybdenum-blue reaction (Tillman and Syers, 1975).

All analyses were done in duplicate and the results reported are averages of duplicates.

Frozen samples were allowed to thaw completely at room temperature before subsampling for analysis.

The average absolute percent variation (AAV) from the initial concentration of any particular nutrient form was calculated from the relationship:

$$AAV = \frac{\left|100\ (1 - \frac{x_1}{x_0})\right| + \left|100\ (1 - \frac{x_2}{x_0})\right| + \ldots + \left|100\ (1 - \frac{x_n}{x_0})\right|}{n.}$$

where x_0 is the initial concentration, x_1 the concentration at the first sampling time, x_2 the concentration at the second sampling time, x_n the concentration at the nth sampling time.

A preservative technique was considered highly satisfactory if the AAV was less than or equal to 5%.

4.4 Results and discussion

4.4.1 Initial characterisation

The concentrations of P and N forms in the freezing works effluent were, with the exception of DNO_3-N, higher than in the sewage effluent sample (Table 4-2). Both effluents initially contained very small amounts of DNO_3-N in a large pool of NH_4-N plus organic-N. The freezing works effluent had a higher proportion of its total N present in organic forms (44%) than did the sewage effluent (17%).

Table 4-2 Initial characterisation of the municipal sewage effluent and freezing works effluent used to evaluate preservation techniques.

Nutrient form	Sewage effluent	Freezing works effluent
	$mg.\ell^{-1}$	
DIP	1.65	3.88
DNH_4-N	14.9	46.3
TNH_4-N	15.0	50.9
DNO_3-N	0.16	0.14
DKN	16.3	65.3
TKN	18.0	90.9

The major inorganic N component in both effluents was NH_4-N. Whereas the sewage effluent had practically all of its NH_4-N present in the dissolved form, almost 10% of the NH_4-N in freezing works effluent was associated with the solid phase, thus justifying the subdivision into DNH_4-N and TNH_4-N.

4.4.2 Behaviour of control treatments

1. Dissolved inorganic phosphorus

The DIP concentration in both effluents declined over the first 4 days of storage (Figure 4-1(A)). In sewage effluent this drop in DIP level was steady, reaching a value 78% of that of the original. Over the remainder of the storage period DIP concentration was almost constant, although 15-20% less than the time zero value.

In the freezing works effluent, DIP increased slightly during the first 2 days of storage. Between day 2 and day 4, however, a large drop in DIP occurred and at day 4 only 21% of the initial value remained (Figure 4-1(A)). After this time, DIP concentration varied with a further peak at day 17 of 79% of the time zero value, declining to 44%

at day 22.

This pattern of rise and fall of DIP is suggestive of periods of microbial growth, with associated declines in DIP due to incorporation into cellular structure. Death of microorganisms due to population density and/or nutrient limitations could be responsible for the release of DIP.

From these results it is clear that some form of preservation technique is required for both effluents if DIP is to be determined at any extended time after sample collection. A rapid removal of DIP from solution can occur in either effluent, although the fluctuations in DIP characteristic of freezing works effluent constitute a greater problem than is the case for sewage effluent.

2. Ammonium-nitrogen

Over 99% of the TNH_4-N in the sewage effluent was in the dissolved form and because of this the unfiltered and filtered samples behaved similarly (Figure 4-1 (B) and (C)). In both the filtered and unfiltered samples the NH_4-N levels remained stable over the first 5 days of storage. Small decreases in NH_4-N concentration occurred between day 5 and day 12, with large losses occurring after day 12, indicative of nitrification (in conjunction with NO_3-N figures). Although at the end of the storage period both the unfiltered and filtered samples contained essentially similar NH_4-N concentrations, the rate of nitrification in the unfiltered sample appeared to be more rapid, as indicated by the substantially lower NH_4-N concentration in that sample at day 22. This is probably due to the larger initial population of nitrifying bacteria; although sufficient nitrifying bacteria must have passed through the membrane filter to seed the filtered sample. Final NH_4-N values were only 3-4% of the initial value.

In the freezing works effluent samples the relatively large amount of NH_4-N associated with the particulate phase (Table 4-2) and the substantial pool of organic-N resulted in some differences in NH_4-N changes between filtered and unfiltered samples. The NH_4-N levels increased markedly during the first 3 days of storage (Figure 4-1 (B) and (C)) in both filtered and unfiltered samples, possibly due to the rapid mineralisation of organic nitrogen compounds. Levels of NH_4-N fluctuated in both sets of samples after

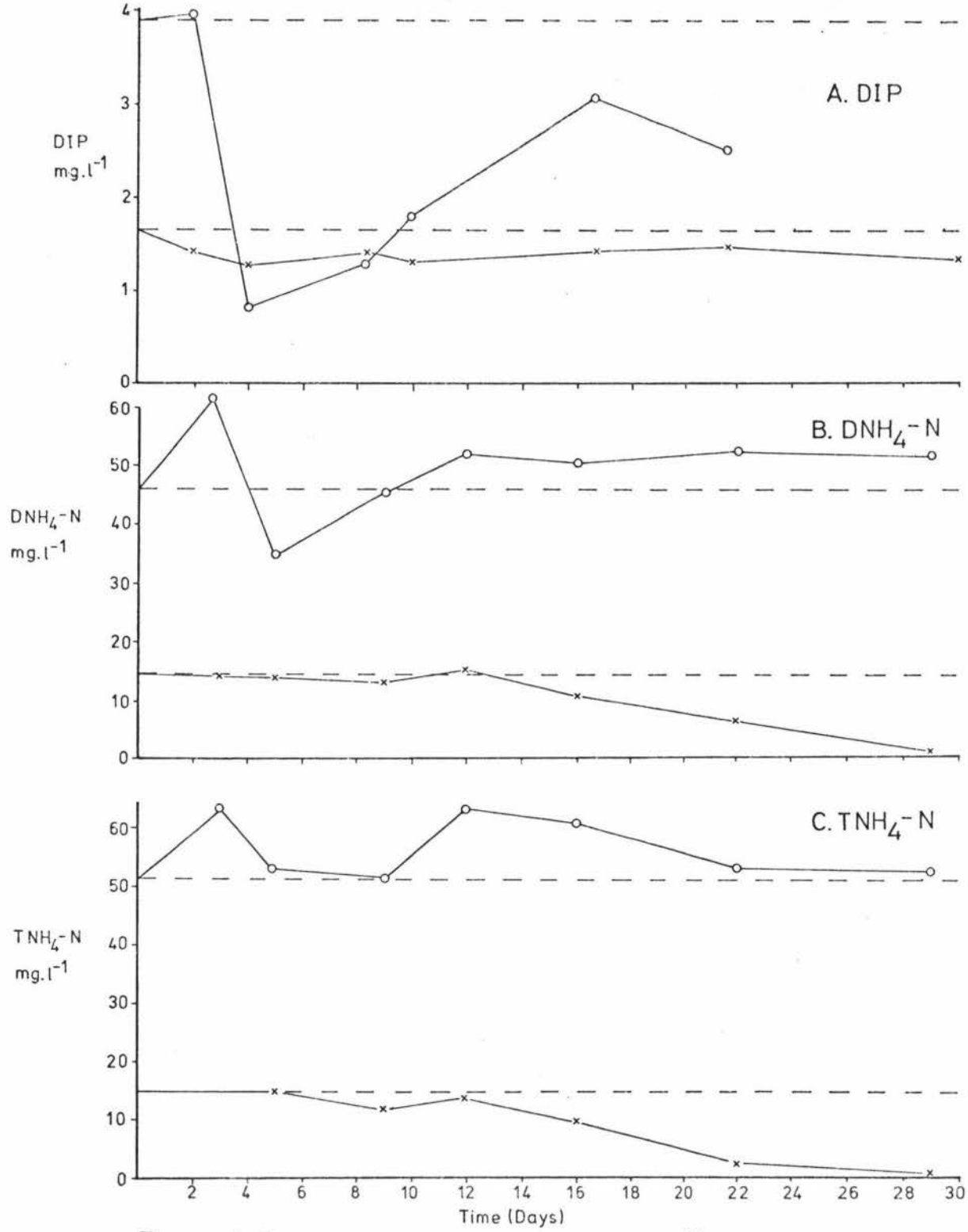


Figure 4-1

Effect of control treatments on concentrations of N and P forms in effluent samples

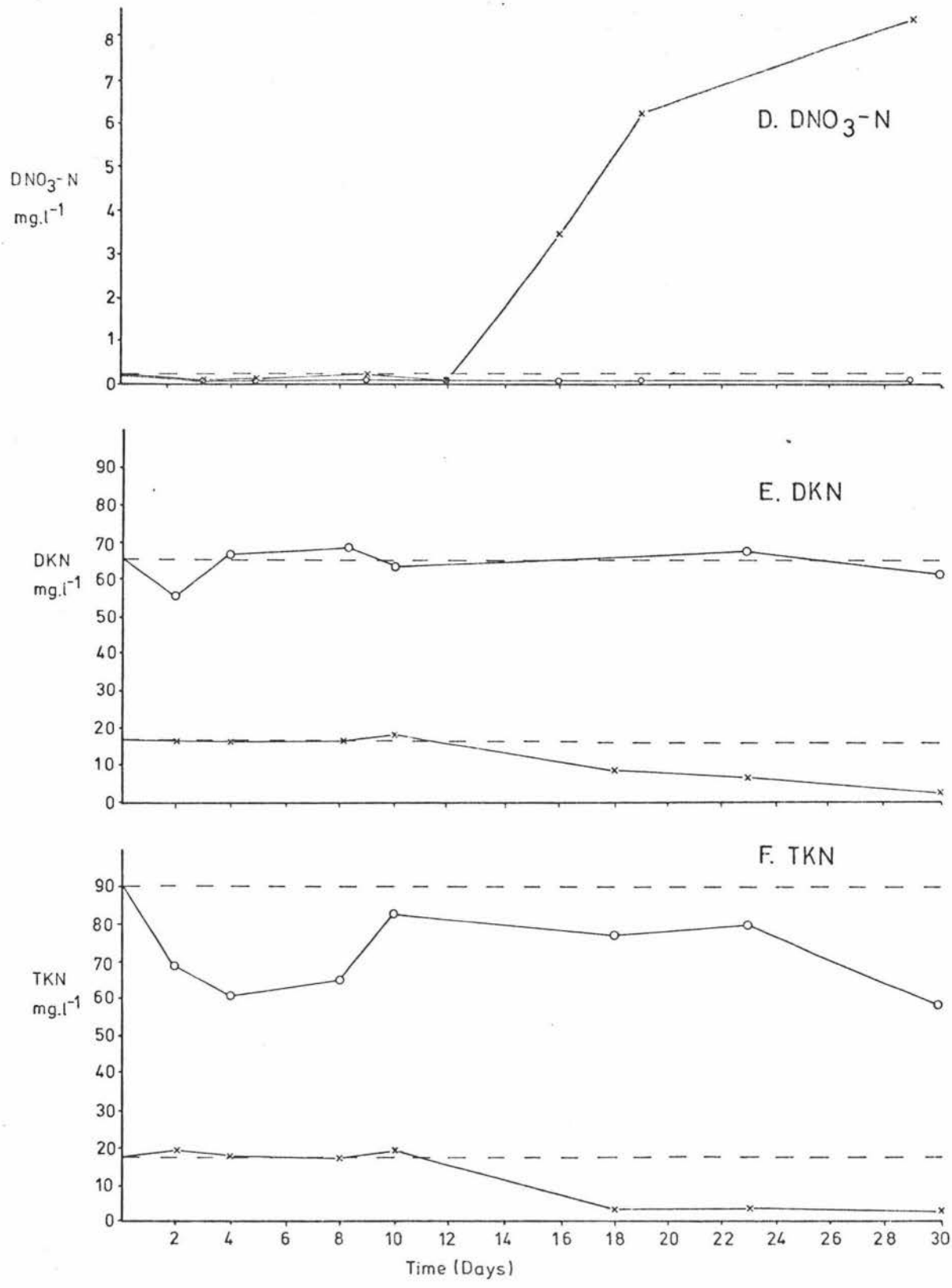


Figure 4-1 continued

this time, with the filtered samples showing the largest decreases (Figure 4-1 (B)) and unfiltered samples the largest increases (Figure 4-1(C)). This difference may be attributed to the fact that in the unfiltered samples the processes of dissolution and mineralisation combatted the microbial utilization of NH_4-N necessitated by the low levels of NO_3-N. The oscillatory behaviour of NH_4-N concentration with time is similar to the pattern obtained for DIP in freezing works effluent.

There was no evidence of any large scale nitrification taking place in the 30-day storage period, as was the case with sewage effluent.

3. Nitrate-nitrogen

The behaviour of NO_3-N in sewage effluent (Figure 4-1 (D)) essentially complemented that of NH_4-N. The NO_3-N concentration remained approximately constant over the first 12 days of storage. After this time NO_3-N increased steadily reaching 8.30 $mg.\ell^{-1}$ after 4 weeks of storage. This rise in NO_3-N was undoubtedly due to nitrification as it was paralleled by a decline in NH_4-N over the same time interval.

The concentration of NO_3-N in freezing works effluent declined rapidly with time of storage and after 3 days a value of 0.02 $mg.\ell^{-1}$ was obtained compared with an initial concentration of 0.14 $mg.\ell^{-1}$. This concentration remained essentially constant throughout the 30-day storage period.

The initial decline in NO_3-N can be attributed to nitrate assimilation by microorganisms and/or denitrification. The possibility of denitrification is enhanced by the anaerobic conditions present, the large supply of readily-available organic substrate, and the neutral to slightly alkaline conditions.

Although net nitrification did not apparently take place during the 30-day storage period, analyses on freezing works samples stored for an additional 2 months after completion of the experiment revealed that NO_3-N concentration had increased to 63 $mg.\ell^{-1}$. This would indicate that virtually all of the original nitrogen substrate had been nitrified.

4. Kjeldahl-nitrogen

As was the case for NH_4-N concentrations, the Kjeldahl-N level

(DKN and TKN) in sewage effluent remained almost constant over the first 10 days of storage. After this time the effects of nitrification became apparent (Figure 4-1(E) and (F)). Similarly, the decline in Kjeldahl-N was more rapid in the unfiltered samples than in the filtered samples.

The high proportions of Kjeldahl-N as NH_4-N and the similarity of behaviour of these two N forms indicate that the preservation difficulties of N forms in sewage effluent are due almost entirely to inorganic-N.

The losses of Kjeldahl-N in freezing works effluent (Figure 4-1 (E) and (F)) over the first 4 days of storage, in conjunction with the increases in NH_4-N (Figure 4-1(B) and (C)), are good evidence that mineralisation of organic-N has occurred. The overall net loss of NH_4-N during this time, as evidenced by the overall drop in Kjeldahl-N, suggests that some NH_4-N must have been lost either by volatilisation or denitrification. This loss is greatest in the unfiltered effluent, probably due to a higher initial bacterial population. In the filtered effluent samples the control treatment was as good as or better than any of the preservative treatments, with respect to holding DKN levels to the initial value. Over the 30-day storage period the average absolute variation (AAV) from the time zero value was only 5.2% (Table 4-3).

The complementary behaviour of Kjeldahl-N and NH_4-N and the absence of demonstrable nitrification, suggest that N-form preservation difficulties in freezing works effluent are likely to be due entirely to mineralisation of organic-N and/or loss of NH_4-N due to volatilisation or denitrification.

4.4.3 Effect of preservative treatments

1. Dissolved inorganic phosphorus

A decrease in temperature of storage had a positive effect in maintaining DIP concentrations close to the initial values in both effluents (Figure 4-2(A) and (B)). Although DIP concentrations declined over the first 4 days at all temperatures, the rate of decline decreased with decreasing temperature. The average absolute variation (Table 4-3) for the unamended effluents decreased with a decrease in temperature, reaching an optimum at -10 C of 5.2% and 3.5% for sewage effluent and freezing works effluent respectively.

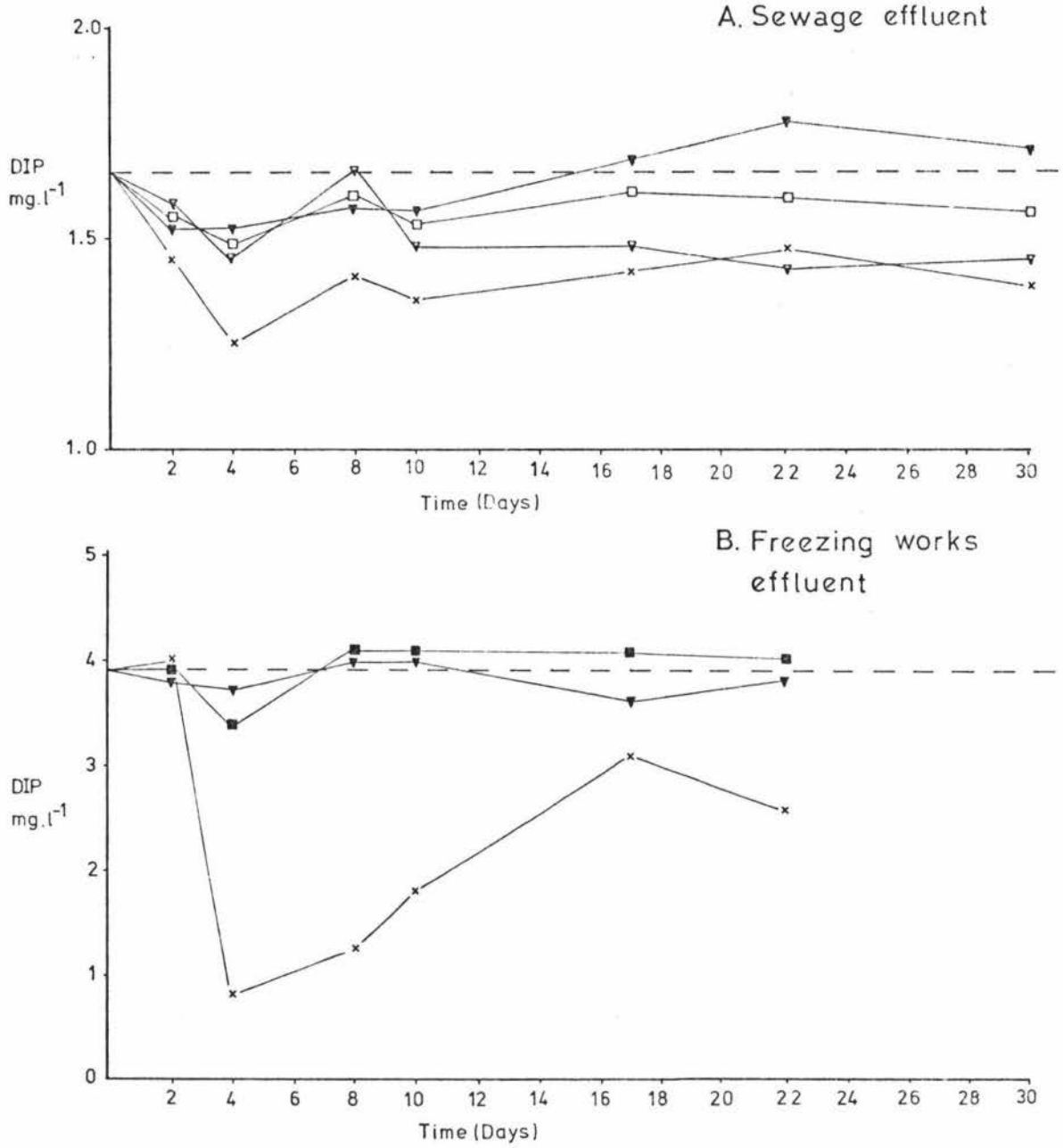


Figure 4-2

Effect of preservative treatments on DIP concentrations in effluent samples

Key:

- Control
- Unamended 4 C
- Unamended -10 C
- $HgCl_2$ Room Temp
- $HgCl_2$ -10 C
- Initial concentration

Table 4-3 Average absolute percent variation (AAV) with respect to initial value of N and P forms in municipal sewage and freezing works effluent over a 30-day storage period under varying preservative treatments

Effluent	Storage temperature	Amendment	Nutrient form					
			DIP	DNH_4-N	TNH_4-N	DNO_3-N	DKN	TKN
			AAV (%)					
Municipal sewage effluent	Room	-	15.4	27.9	40.0	1611	28.4	37.7
	Room	N-Serve	18.1	8.9	8.0	132	14.4	4.7
	Room	$HgCl_2$	5.1	7.9	8.4	33.9	5.8	8.8
	4 C	-	8.2	6.0	4.4	48.2	2.8	6.5
	4 C	N-Serve	16.4	7.9	9.4	29.4	5.6	3.8
	4 C	$HgCl_2$	5.4	7.9	7.4	47.3	2.9	8.1
	-10 C	-	5.2	4.6	9.3	53.5	8.6	8.3
	-10 C	N-Serve	7.1	21.1	18.3	48.2	22.5	14.4
	-10 C	$HgCl_2$	7.1	18.4	10.0	59.8	22.5	9.9
Freezing works effluent	Room	-	44.7	15.4	13.1	78.6	5.2	22.6
	Room	N-Serve	37.4	11.9	15.6	60.7	7.7	17.1
	Room	$HgCl_2$	15.4	9.1	11.8	13.1	8.8	17.1
	4 C	-	22.7	8.5	7.3	57.1	6.0	12.8
	4 C	N-Serve	24.8	7.3	8.9	60.9	5.6	8.9
	4 C	$HgCl_2$	10.3	5.2	4.9	41.6	5.5	9.8
	-10 C	-	3.5	10.2	11.1	48.8	13.2	20.4
	-10 C	N-Serve	10.7	16.7	11.1	51.2	15.7	20.7
	-10 C	$HgCl_2$	4.2	4.4	6.6	61.9	15.8	25.2

Addition of a preservative at any storage temperature did not improve on the preservation of DIP which was achieved by freezing alone.

As was expected the addition of N-Serve did not improve DIP preservation at any temperature (Appendix 2-1). The trends observed in the unamended effluents were essentially paralleled by the N-Serve treatments.

The addition of $HgCl_2$ to samples maintained at room temperature, however, greatly improved the stability of DIP concentrations in both effluents, but more particularly in the municipal sewage (Figure 4-2). Decreasing the storage temperature of the $HgCl_2$-amended samples did not lead to further improvement in DIP preservation in sewage effluent (Table 4-3). In freezing works effluent, however, storage at -10 C improved the stability to within 5% of the initial value.

2. Ammonium-nitrogen

A decrease in the temperature of storage was sufficient to stabilise NH_4-N concentration in both filtered and unfiltered samples of sewage effluent (Figure 4-3). Storage at 4 C was superior to freezing with unfiltered samples (Figure 4-3(A)), whereas storage at -10 C was slightly superior with the filtered samples (Figure 4-3(B)). The addition of a preservative and storage at any temperature proved to be inferior to the above treatments. The N-Serve amendment inhibited the nitrification observed in the control treatment (Section 4.2.2) for up to 22 days (Appendices 2-2 and 2-3) but after this time losses of NH_4-N occurred at room temperature. Samples of this treatment analysed 2 months after completion of the experiment revealed that NH_4-N concentration had dropped to 1.1 mg.ℓ^{-1} compared with the initial concentration of 15 mg.ℓ^{-1}, indicating that the NH_4-N had been nitrified almost completely. A decrease in the storage temperature of samples containing N-Serve or $HgCl_2$ resulted in large losses of NH_4-N in both filtered and unfiltered samples (Appendices 2-2 and 2-3). This loss increased with decreasing storage temperature with the maximum loss occurring in the frozen, N-Serve-amended treatments (Figure 4-3).

In contrast to sewage effluent, decreasing the storage temperature of unamended freezing works samples did not stabilise NH_4-N concentrations (Appendices 2-2 and 2-3). Amendment with $HgCl_2$ and storage at either 4 C or -10 C proved to be the best method of preservation for both unfiltered (Figure 4-4(A)) and filtered samples (Figure 4-4(B)).

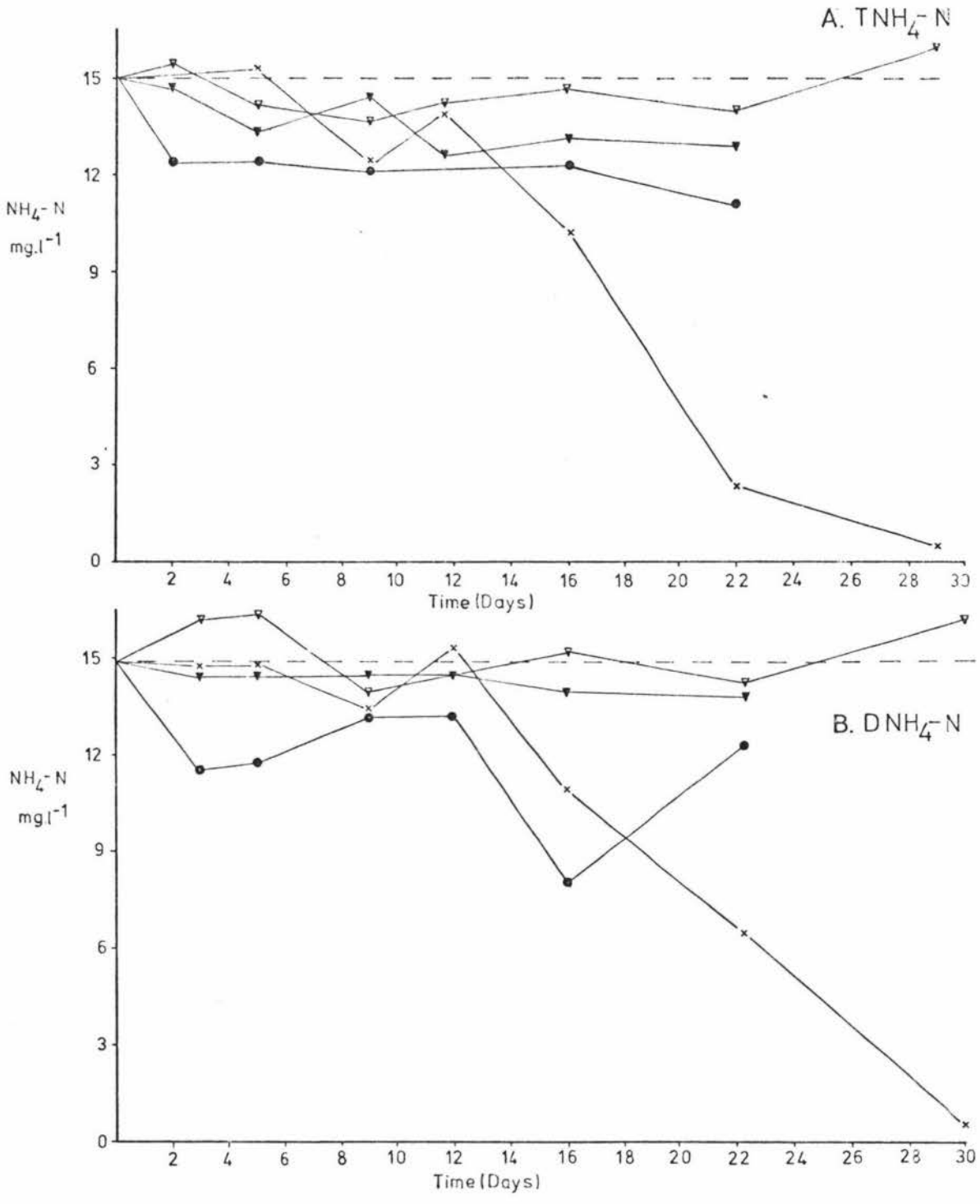


Figure 4-3

Effect of preservative treatments on NH_4-N concentrations in sewage effluent samples

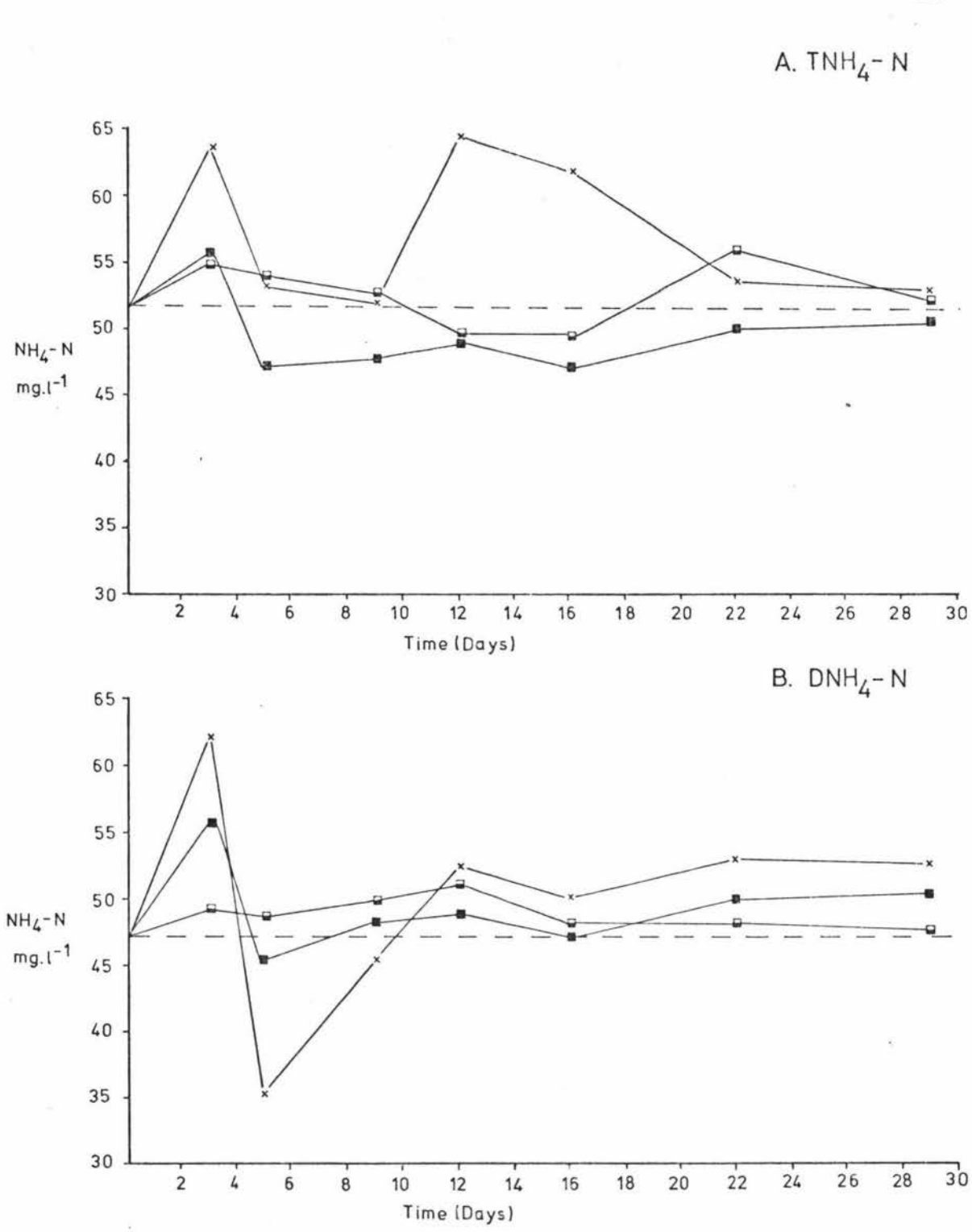


Figure 4-4

Effect of preservative treatments on NH_4-N concentrations in freezing works effluent samples.

Key:

- Control
- $HgCl_2$ 4 C
- $HgCl_2$ -10 C
- Initial concentration

In both filtered and unfiltered $HgCl_2$-amended samples an initial large increase in NH_4-N was observed within the first 3 days of storage. The same trend occurred in the control treatments (Section 4.3.2). The smaller increase in NH_4-N in the $HgCl_2$-treated effluent may have occurred in the time between the initial analysis and the addition of $HgCl_2$ to the subsample (approximately 1½ hours).

For both effluents, the effect of freezing of the unamended, filtered effluent was not significantly different from storage at 4 C with respect to the preservation of NH_4-N. For the unfiltered, unamended effluents, however, storage at 4 C led to better preservation than storage at -10 C. Thus it would appear that freezing of particulates increased the variability of NH_4-N concentrations.

3. Nitrate-nitrogen

For both effluents room temperature storage with $HgCl_2$ appeared to be the best method of preservation of NO_3-N (Figure 4-5). Storage of N-Serve-amended sewage effluent samples at 4 C gave slightly less variable results for NO_3-N than did storage with $HgCl_2$ at room temperature (Figure 4-5(A)). The latter treatment was greatly superior for freezing works effluent (Figure 4-5(B)).

Decreasing the storage temperature decreased the recovery of NO_3-N in sewage effluent, both with unamended (Figure 4-5(A)) and amended samples. In contrast, a decrease in the temperature of storage of unamended freezing works effluent tended to increase the recovery of NO_3-N (Figure 4-5(B)).

Although room temperature storage of $HgCl_2$-amended samples is considered to be the best preservative for NO_3-N in this experiment, even this treatment exceeded the 5% average absolute variation (Table 4-3) which was considered desirable. There are two interrelated factors which undoubtedly influence this variability. First, in both effluents only a small concentration of NO_3-N was initially present in a large pool of NH_4-N + organic-N. Thus, even small changes in the latter pool would cause a large percentage change in NO_3-N levels. Secondly, the analytical error in the method used for NO_3-N in this experiment is likely to be of greater magnitude than the other nutrient forms under study (Chapter 3).

4. Kjeldahl-nitrogen

A decrease in the storage temperature of unamended effluent to 4 C

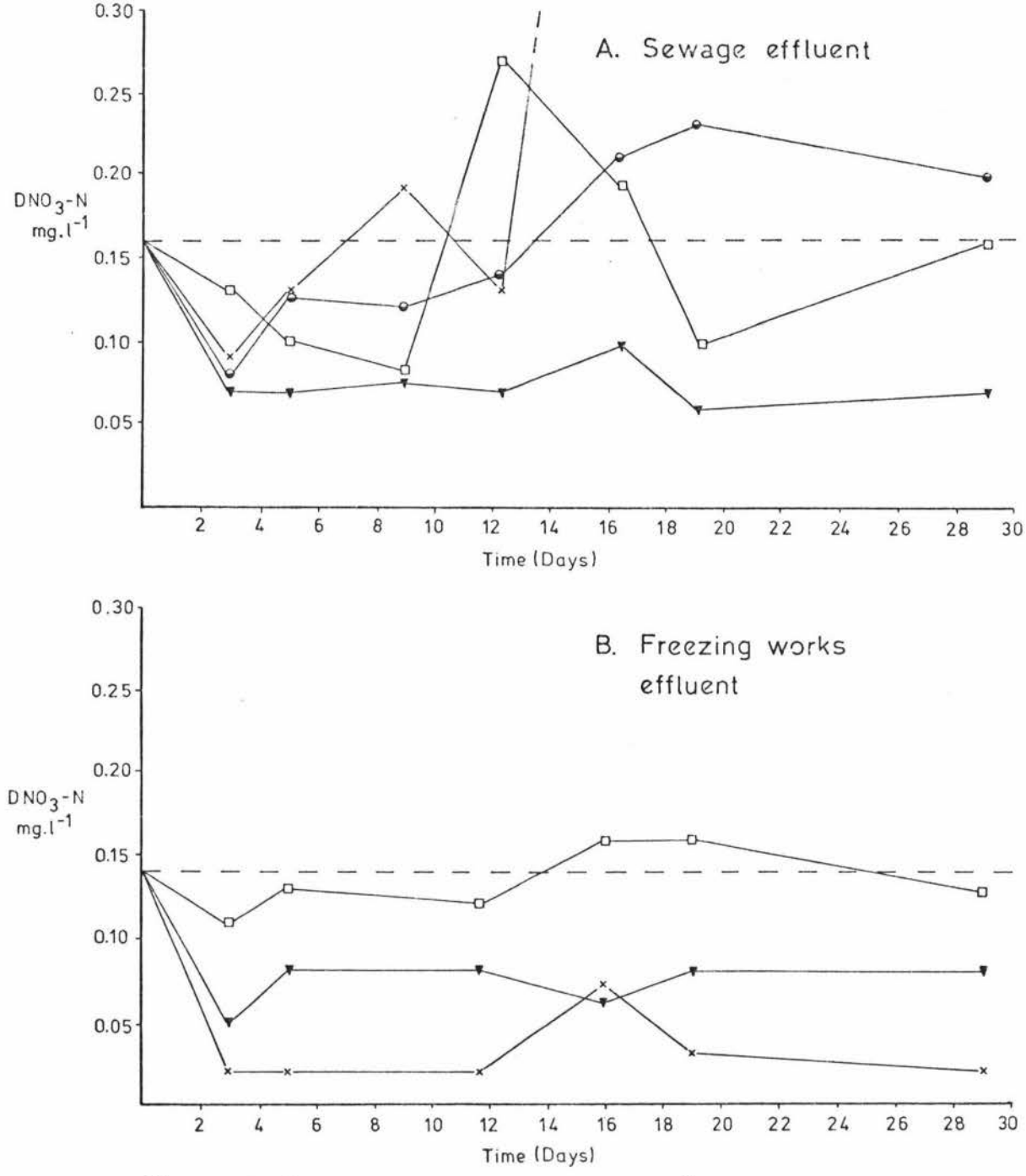


Figure 4-5

Effect of preservative treatments on DNO_3-N concentrations in effluent samples

proved to be the most effective form of preservation for DKN in sewage effluent (Figure 4-6(B)), and reduced the AAV to an acceptable 2.8%. The effect of the same treatment on filtered freezing works effluent is more difficult to evaluate because no significant differences existed between the control treatment and the three best DKN preservative treatments (Table 4-3). Temperature control, on its own, did not adequately preserve TKN in either effluent.

Amendment with N-Serve and storage at 4 C appeared to be the most successful method of preservation of TKN in both effluents (Figures 4-6(A) and 4-7(A)). This result must be treated with caution, however, as there is an apparent anomally between the Kjeldahl-N and the NH_4-N results. The addition of N-Serve did not prevent the decreases in NH_4-N observed with the unamended effluents. It is therefore suspected that the apparent beneficial effects of N-Serve in Kjeldahl-N preservation are due to decomposition of N-Serve itself during the Kjeldahl digestion, compensating for a loss of NH_4-N. An alternative explanation could be that the N-Serve is retarding or halting mineralisation of organic-N and therefore subsequent gaseous-N losses. This may account for the more pronounced effect of N-Serve on the unfiltered samples which probably had a higher proportion of organics.

Amendment with $HgCl_2$ had diverse effects on the preservation of Kjeldahl-N in effluent samples. In freezing works effluent, amendment with $HgCl_2$ did not improve either DKN or TKN preservation, whereas in sewage effluent $HgCl_2$ addition improved the preservation of DKN at both room temperature (Figure 3-6(B)) and 4 C (Appendix 2-5). Amendment of unfiltered sewage stored at room temperature caused another anomally. All the TKN values given by this treatment (Figure 4-6(A)) were higher than the initial time zero value. This may be explained by the possible addition of the $HgCl_2$ to the unfiltered subsample slightly before (1 to 2 hours at the most) the time zero analysis for TKN. Unfortunately, no data were obtained for time intervals between preservative addition and the initial analysis. Despite the above anomally, amendment with $HgCl_2$ and storage at room temperature was second only to treatment with N-Serve as a preservative technique for TKN in sewage effluent.

Kjeldahl-N values (DKN and TKN) for $HgCl_2$-amended sewage samples were not higher than the time zero value at all storage temperatures. It appears that some loss of Kjeldahl-N occurs when the storage

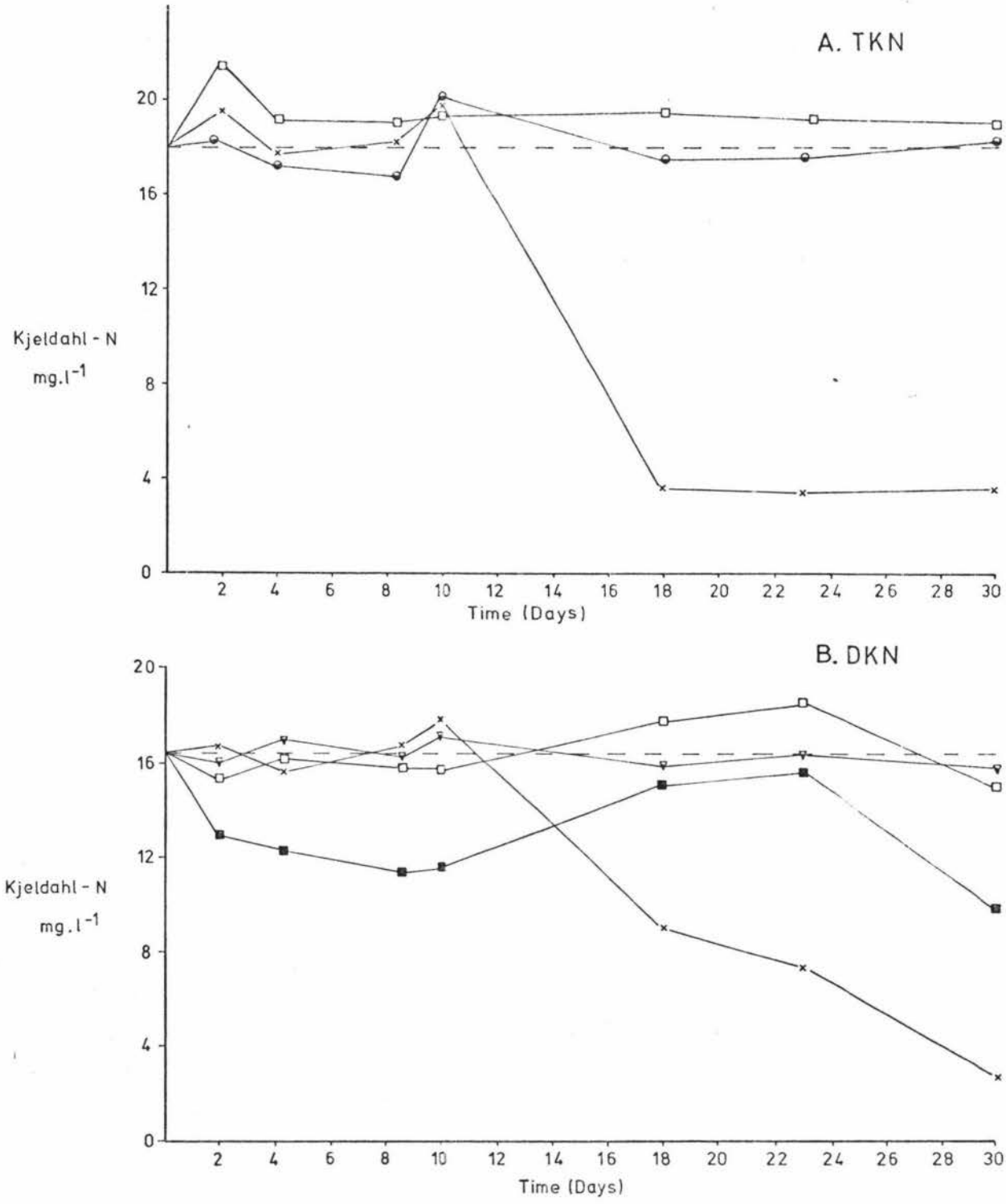


Figure 4 - 6

Effect of preservative treatments on TKN and DKN concentrations in sewage effluent samples.

Key:

- Control
- Unamended 4 C
- N-Serve 4 C
- $HgCl_2$ Room Temp
- $HgCl_2$ -10 C
- Initial concentration

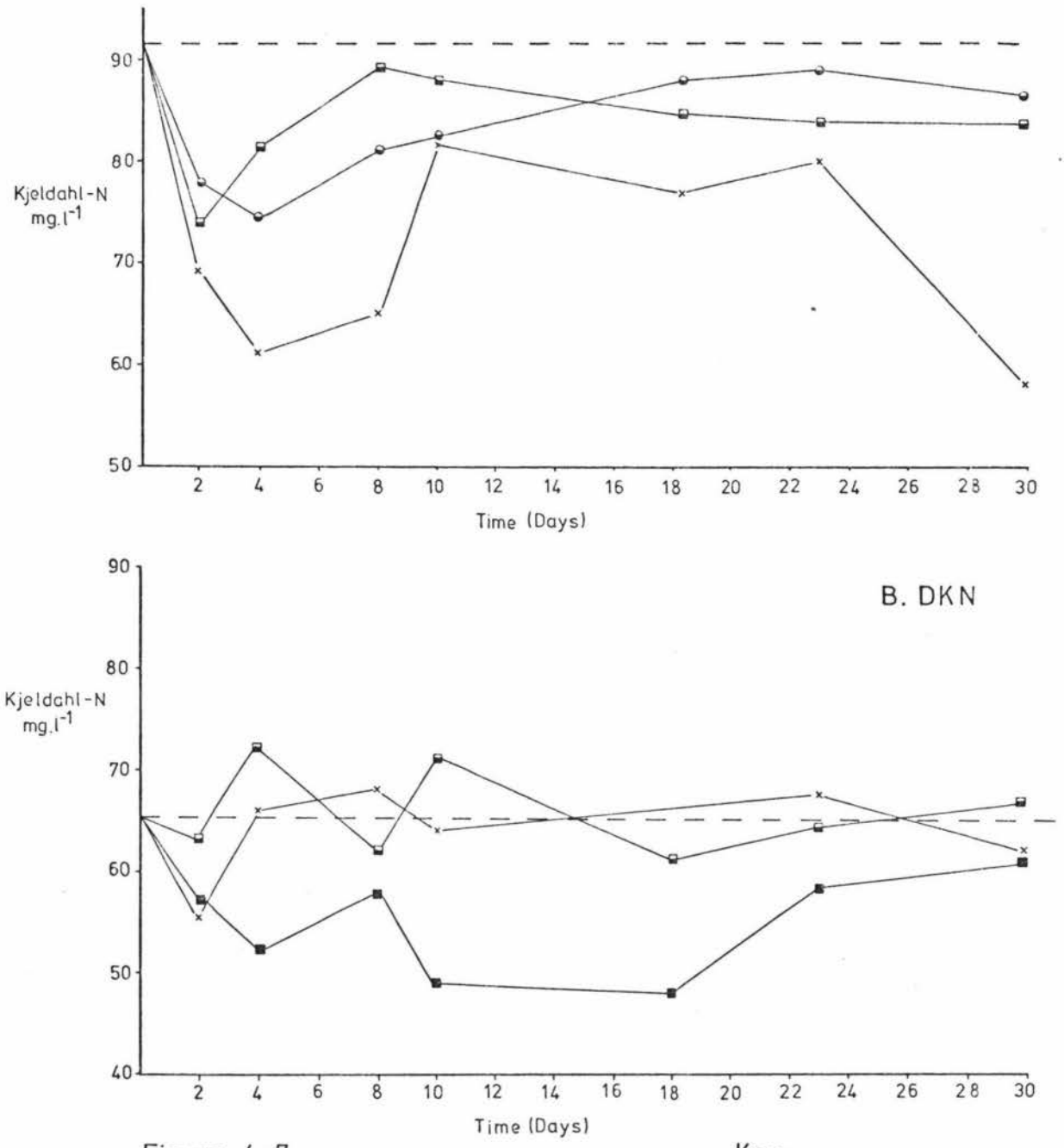


Figure 4-7

Effect of preservative treatments on TKN and DKN concentrations in freezing works effluent samples

temperature is lowered below freezing point. The effect is common to all -10 C treatments in both effluents but is enhanced when the effluent has a preservative amendment (Appendices 2-5 and 2-6). Freezing $HgCl_2$-amended filtered samples resulted in the greatest losses (Figures 4-6(B) and 4-7(B)). It is likely that the apparent losses in Kjeldahl-N are due to the NH_4-N component because similar losses of this nutrient form were obtained.

4.5 General discussion and recommendations

4.5.1 Dissolved inorganic phosphorus

Comparisons between the results obtained in this study and other studies where preservation of DIP has been considered are difficult for two reasons:

(1) Work in this field has been done almost exclusively on freshwater or seawater samples that contain low concentrations and have low biological activity; and

(2) A different approach to pretreatment of samples before commencement of storage has been used.

Workers concerned with the preservation of DIP in runoff or stream samples (Nelson and Römkens, 1972; Klingaman and Nelson, 1976; Johnson *et al.*, 1975) chose to store their samples in an unfiltered condition and to centrifuge and filter the samples prior to analysis. This approach was rejected for the effluents used in the present study because of; their (1) high biological activity, and (2) high suspended solids content.

Despite these obvious differences, certain comparisons are possible. The behaviour of the control treatments bears some resemblance to the results of Klingaman and Nelson (1976) who found that DIP concentrations stored at 23 C without amendment tended to stabilise after an initial decrease. In the present experiment DIP concentrations in the control treatments for both effluents reached a minimum after 4 days. Instead of stabilising after this time, however, the concentrations fluctuated in a manner suggestive of microbial growth and decline. This is undoubtedly a reflection of the higher biological activity in the effluent samples.

The use of $HgCl_2$ as a preservative, in combination with low temperature storage, has been recommended as a suitable preservative technique for DIP in runoff and stream samples (Klingaman and Nelson, 1976; Goulden, 1972), and estuarine samples (Jenkins, 1968). The results of this study suggest that $HgCl_2$ amendment with subsequent storage at either room temperature or 4 C is a good preservative technique for DIP in filtered sewage effluent, but that the freezing of $HgCl_2$-amended samples is necessary for satisfactory DIP preservation in freezing works effluent. Comparison of the results for unamended and $HgCl_2$-amended samples would suggest that lowering the storage

temperature and addition of $HgCl_2$ exerted approximately an equal influence in preserving the DIP levels in sewage effluent but that lowering the temperature below freezing point was the dominant influence in freezing works effluent.

The data obtained in this experiment would suggest that no advantage is gained by addition of $HgCl_2$ over freezing of unamended, prefiltered samples.

4.5.2 Nitrogen forms

The results of this study indicate that the best general preservative method for N forms in sewage effluent is unamended storage at 4 C. For freezing works effluent, the best method is $HgCl_2$ amendment and storage at 4 C. The exception for both of these effluents is DNO_3-N, for which $HgCl_2$ amendment and storage at room temperature was the most satisfactory method. The need for $HgCl_2$ amendment in freezing works effluent, but not in sewage effluent, may be explained by the higher proportion of organic and particulate N in freezing works effluent (see Chapter 6). This result is in agreement with the work of Hellwig (1967) who recommended increasing the amount of $HgCl_2$ when increasing amounts of organics were present in the effluent.

The 890 $mg.\ell^{-1}$ $HgCl_2$ used by Hellwig (1967) for preserving raw sewage effluent would not be required for the effluents used in this experiment. A combination of 50 $mg.\ell^{-1}$ $HgCl_2$ and 4 C storage provided adequate preservation for both effluents. No evidence of any interference with ammonia distillation was found at this concentration of $HgCl_2$; a result in agreement with Howe and Holley (1969). The use of $HgCl_2$ at 50 $mg.\ell^{-1}$, with subsequent refrigeration, for the preservation of N forms in effluents is in accord with the findings of others for the preservation of N forms in natural and polluted waters (Jenkins, 1968; Howe and Holley, 1969; Goulden, 1972; Klingaman and Nelson, 1976). These authors all reported that 40-50 $mg.\ell^{-1}$ $HgCl_2$ and storage at 4 C was satisfactory for preservation of various N forms.

An interesting observation was that freezing proved to be inferior to storage at 4 C for all nitrogen forms except NH_4-N in filtered samples. These results indicate that freezing is an inappropriate method of storage where the sample; (i) contains particulate matter, and (ii) where an organic component is to be analysed. This result is

difficult to compare with others in the literature as invariably the dissolved component is obtained by filtering a previously unfiltered sample after storage.

The large losses of NH_4-N and Kjeldahl-N which occurred upon freezing with certain treatments is difficult to explain. No other such occurrence has been reported in the literature, with the exception of Jenkins (1968) who noted large losses of NH_4-N on freezing unamended estuarine water for 30 days. The losses of Kjeldahl-N on freezing appear to reflect a loss of the NH_4-N component. Also, the loss of NH_4-N on freezing appears to be strongly correlated with the addition of a preservative amendment, the one exception being $HgCl_2$-amended freezing works samples stored at -10 C. The concentration gradient theory of Nelson and Römkens (1972), and Omang and Vellar (1973), while having superficial attraction, must be discounted for two reasons; (i) the bottles were shaken before subsampling for analysis, and (ii) similar losses were not noted for DIP. Clearly, a theory involving gaseous loss of NH_4-N must be invoked. Gaseous losses of NH_4-N in aqueous systems are brought about by either chemical or microbiological mechanisms. Of these two the latter would seem unlikely. Although rapid genesis of microorganisms is possible after thawing a frozen sample, it is implausible that this would be encouraged by addition of a physiological suppressent. Therefore losses by chemical mechanisms seem the most likely. Ammonia volatilisation will occur under conditions of alkaline pH. It may be possible that concentration gradients in the thawing effluent brings about a localised concentration of $NH_4^+{}_{(aq)}$ and $OH^-{}_{(aq)}$ ions, thereby raising the pH, and allowing $NH_3{}_{(g)}$ to volatilise.

4.5.3 Recommendations

A summary of the best preservative treatments appropriate for each effluent and for each of the analytical parameters (Table 4-4) supports the view of the majority of workers (APHA, 1971) that no one preservative treatment is completely satisfactory. In general it would appear that freezing is the best treatment for dissolved inorganic forms but is not suitable for organics or for particulates. For samples with a high organic content where conversions from the organic to inorganic forms (and vice versa) may present problems, the addition of $HgCl_2$ will help in preservation. For the two effluents in this study the best overall preservative treatments are; freezing and refrigeration for

Table 4-4 Storage treatments appropriate for the long-term (30 days) preservation of N and P forms in effluent samples.

	Sewage effluent		Freezing works effluent	
	Best method	Other satisfactory methods	Best method	Other satisfactory methods
Nutrient form				
DIP	Unamended -10 C	$HgCl_2$ room temperature $HgCl_2$ 4 C	Unamended -10 C	$HgCl_2$ -10 C
DNH_4-N	Unamended -10 C	Unamended 4 C	$HgCl_2$ -10 C	$HgCl_2$ 4 C
TNH_4-N	Unamended 4 C		$HgCl_2$ 4 C	$HgCl_2$ -10 C
DNO_3-N	N-Serve 4 C	$HgCl_2$ room temperature	$HgCl_2$ room temp.	
DKN	Unamended 4 C	$HgCl_2$ 4 C	Unamended room temperature	$HgCl_2$ 4 C N-Serve 4 C*
TKN	N-Serve 4 C*	N-Serve room temp.* Unamended 4 C $HgCl_2$ room temperature $HgCl_2$ 4 C	N-Serve 4 C*	$HgCl_2$ 4 C

*This may be a false result as discussed in Section 4.3.5.

filtered and unfiltered samples respectively, with the addition of $HgCl_2$ to freezing works samples prior to such storage being useful.

CHAPTER 5 GROWTH AND DIE-OFF PATTERNS OF INDICATOR BACTERIA IN EFFLUENT SAMPLES

5.1 Introduction

In planning a sampling programme for the characterisation of effluents, it is important to know that both quantitative and qualitative changes in the composition of the effluent samples will be kept to a minimum prior to sample analysis. This is particularly true for a bacteriological sampling programme, where some methods for holding the bacterial population at equilibrium must be used.

Such methods are well established for natural water samples, the most common being cooling of the sample below 10 C (APHA, 1971). The use of holding media has also been mooted (Millipore, 1973) where a delay of greater than 6 hours may be incurred between sampling and analysis.

Similar recommendations for effluents are lacking with only sewage effluent (Silvey *et al.*, 1974) receiving much attention in this regard due to its public health significance.

As characterisation studies normally involve 24 hour sampling, the use of some form of automatic sampling equipment is virtually a necessity. The use of such equipment precludes the use of holding media. Therefore the storage of effluents at ambient temperature and 4 C were selected for evaluation as pre-analysis methods for the enumeration of indicator bacteria in effluent samples.

5.2 Review of Literature

The survival of indicator bacteria in effluents has received much less attention than similar phenomena in seawater or freshwater situations. The exception to this is municipal sewage effluent in which wastewater engineers have been interested in the regrowth of different bacteria and viruses following chlorination of the sewage (Shuval *et al.*, 1973; Silvey *et al.*, 1974).

In field studies on natural waters the survival of indicator bacteria has been shown to be inversely proportional to temperature between 0 and 15 C (Kittrell and Furfari, 1963; Geldreich *et al.*, 1968; McFeters and Stuart, 1972; Davenport *et al.*, 1976). The latter authors reported that maximum survival of indicator bacteria under natural conditions occurred in 0 C water under ice.

Under laboratory conditions the relationship of temperature with survival of bacteria has been shown to be dependent on the degree of 'pollution' of the sample (England and Wales Public Health Laboratory Service, 1952; Lonsane *et al.*, 1967). The latter authors found that for 'marginally contaminated' waters the icing of samples did not significantly increase or decrease recovery of coliform organisms, whereas with 'highly polluted' samples significant decreases in numbers of coliforms occurred with time, and icing assisted in reducing this decrease. This observation was explained in terms of competitive and antagonistic effects due to absolute and relative densities of coliform and non-coliform organisms. Lonsane *et al.*, (1967) demonstrated the effect of reducing the competition/antagonism by introducing a pure culture of *E. coli* to an autoclaved polluted sample. Under these conditions *E. coli* multiplied rapidly. The England and Wales Public Health Laboratory Service (1952) observed that coliforms die off more rapidly in highly polluted waters because of the greater ratio of non-coliform organisms to coliforms and because the coliforms in such waters are less acclimatized. These findings are consistent with those discussed by Kittrell and Furfari (1963) on the survival of coliform bacteria on discharge of wastewater into a river. They reported that once sewage effluent is diluted by the river the numbers of coliforms increased until a maximum density was reached approximately one-half day below the point of discharge. At this point the population of predators such as protozoa increased to sufficient numbers to restore the predator-bacteria balance, and a net decline in coliforms resulted.

Although nutrient concentration undoubtedly affects the survival of indicator bacteria in that high populations are usually associated with highly polluted waters, which in turn usually have high nutrient concentrations; the relationship is not a simple one. By inference from the previous paragraph it would appear that the density of coliform bacteria decreases more rapidly when initial nutrient levels are relatively high (Kittrell and Furfari, 1963). However, no significant difference in survival rate related to the possible effects of varying concentrations of nutrient species in stormwater was reported by Geldreich *et al.* (1968). In survival experiments on water samples from a stock dam, Pyle (1974) showed that in unfiltered samples containing the natural population of algae and protozoa, adding nitrate did not stimulate growth, whereas phosphate at 4 $mg.\ell^{-1}$ stimulated indigenous bacterial growth. In samples filtered to remove most algae and protozoa both nitrate and phosphate additions stimulated growth particularly when both nutrients were added. Thus it would seem that in natural populations the effects of nutrients on survival is complex and is undoubtedly associated with other factors such as physiological and environmental conditions (Klock, 1971).

Sediment concentration is one such environmental condition. In a river system bacteria may be adsorbed by sediments and carried to the river bottom (Kittrell and Furfari, 1963; Canale *et al.*, 1973). Thus their recovery from river water will decrease though their absolute survival rate may not have. In seawater samples, the survival of *E. coli* has been found to increase when sediment is present. Seeded sterilised samples taken from an area receiving domestic wastes increased more rapidly when sediments were present (Gerba and McLeod, 1976). This they attributed to higher organic matter in the samples containing sediment.

Other factors to affect indicator bacterial survival are pH (Kittrell and Furfari, 1963), Eh (Klock, 1971), algal or other toxins (Klock, 1971; Canale *et al.*, 1973), and shortwave radiation (Gameson and Saxon, 1967). The optimum pH for growth of coliform bacteria is in the vicinity of 7.0. pH values greater than 8.5 have been reported to cause a ten-fold reduction in coliform density (Kittrell and Furfari, 1963).

All of the factors mentioned above may affect any species of bacteria in some way. It is important to select an indicator bacterium

with survival characteristics in the medium in which they are sampled, which are as close as possible to the pathogen of which they are indicative (McFeters and Stuart, 1972). These authors commented that faecal coliforms may not be useful indicator bacteria because they have exhibited one of the most rapid declines in natural waters of all microorganisms of public health significance. In a later paper McFeters *et al.* (1974) reported that as a group, coliform bacteria died more rapidly than enterococcus groups in wellwater. In a recent study Davenport *et al.* (1976) showed that under iced river conditions, faecal streptococci survived longer than faecal and total coliforms. Similar conclusions have been reached by Miura (1971) and Geldreich *et al.* (1968). The latter authors reported that in stormwater samples, *Streptococcus faecalis* persisted longer and at higher levels than *Aerobacter aerogenes* (a faecal coliform) or, *Salmonella typhimurium*.

Although faecal streptococci may persist for long periods in polluted waters, they generally do not multiply (Geldreich and Kenner, 1969). In a recent study on the long-term survival (up to 7 days) of indicator bacteria in dairy shed effluent samples taken from the same discharge used in the present study, Guy and Small (1977) reported an 80% drop in faecal streptococci numbers in 48 hours for samples stored at 20 C. Faecal coliform numbers increased by more than 100% in the same period. In contrast to the commonly held view (Geldreich and Kenner, 1969; McFeters *et al.*, 1974) that *Streptococcus bovis* and *Streptococcus equinus* (dominant cocci in faeces of cattle) die off very rapidly outside the intestinal tract of the animal, Guy and Small (1977) reported that 18-55% of the *S. bovis* numbers originally isolated in the dairy shed effluent samples, were still being maintained after 4 days, at a range of different storage temperatures (5 C, 10 C, 15 C and 20 C).

From this review of the literature, it can be seen that the factors affecting the survival of various indicator bacteria are numerous and complex. Of the factors discussed, only lowering of temperature appears to be a practical method for controlling the stability of indicator bacterial populations in effluent samples.

5.3 Materials and Methods

At each of the three effluent sources under study (sewage, freezing works, and dairy shed) a grab sample of effluent was taken in a sterile 1 litre Erlenmeyer flask. After shaking to ensure adequate mixing, subsamples were poured into two sterile 270 cm^3 bottles with rubber lined aluminium tops. One of these bottles was immediately placed in an insulated 'chilly bin' containing ice and the other was left at air temperature. The samples were then transported back to the laboratory.

The delay between the time of sampling and the time of the first filtration was approximately 10 minutes for dairy shed effluent, 20 minutes for sewage effluent, and 30 minutes for freezing works effluent.

At the laboratory the chilled sample was transferred to a refrigerator and stored at 4 C.

After time periods of 0, 3, 6, 9, 12 and 24 hours in the laboratory, subsamples were filtered and analysed for total coliform bacteria, faecal coliform bacteria, and faecal streptococci according to the procedures set out in Chapter 3. For each group of indicator bacteria, 3 dilution series were employed in order to cover adequately the expected range. Filtrations at each dilution were done in triplicate and results reported are averages of triplicate analyses.

The time periods described above were chosen after a preliminary experiment indicated that 24 hours was the longest time effluent samples should reasonably be stored prior to analysis.

The doubling time (G) was calculated from the relationship:

$$\frac{\log_{10}2}{G} = \mu = \frac{\log_{10}N_t - \log_{10}N}{t}$$

where t = time, N_t = number after time t, N = initial number, μ = specific growth rate (Meynell and Meynell, 1970).

The doubling time G, is the time taken for a culture to double in concentration or mass. A negative doubling time indicates a net fall in count. It should be remembered that during periods of net decline division is probably continuing but the viable count falls because the death rate exceeds their division rate. Where the doubling time/half life exceeds $\pm 10^2$ hours, G is given as ∞, i.e., the population is essentially stable within the limits of the method used.

Due to lack of data points between 12 and 24 hours, G is calculated over the first 12 hours of incubation only.

5.4 Results and Discussion

The growth and die-off pattern for total coliform bacteria was similar in samples of all three effluents (Figure 5-1). At room temperature all effluent samples showed an increase in coliform numbers over the first 12 hours. The rate of increase is reflected in the mean doubling time (Table 5-1) which increased in the order; sewage < dairy shed < freezing works. Although the factors affecting growth rate are complex, the low growth rate in the freezing works effluent may have been due to the slightly alkaline pH of 7.8 (sewage, pH 7.0; dairy shed effluent, pH 6.8).

Table 5-1 Mean doubling time, G (in the 12 hours after collection) of indicator bacterial groups in effluent samples stored at room temperature and 4 C.

	Sewage effluent		Dairy shed effluent		Freezing works effluent	
	Room	4 C	Room	4 C	Room	4 C
	hours					
Total coliforms	4.6	-20.6	6.9	-8.3	13.3	∞
Faecal coliforms	14.0	-9.8	9.3	-6.4	-17.6	-13.2
Faecal streptococci	∞	∞	2.7	12.6	-12.2	∞

Storage at 4 C slowed division of cells in all effluents to the point where there was a net die-off. The long negative doubling time in the 4 C stored sewage effluent and the obvious stability of the 4 C stored freezing works effluent indicated that these two effluents may be held for up to 12 hours at 4 C before analysis for total coliform bacteria.

Faecal coliforms behaved similarly though more erratically (Figure 5-2) than did total coliforms. In the samples stored at room temperature the mean doubling time in all effluent samples was longer than that of total coliforms. Faecal coliforms in the sewage and dairy shed effluent had similar mean doubling times although their respective growth patterns were markedly different (Figure 5-2). Faecal coliforms in freezing works effluent slowly declined in numbers during the 12 hour storage period. This may be related to the slightly alkaline pH of the effluent though other factors such as physiological condition of

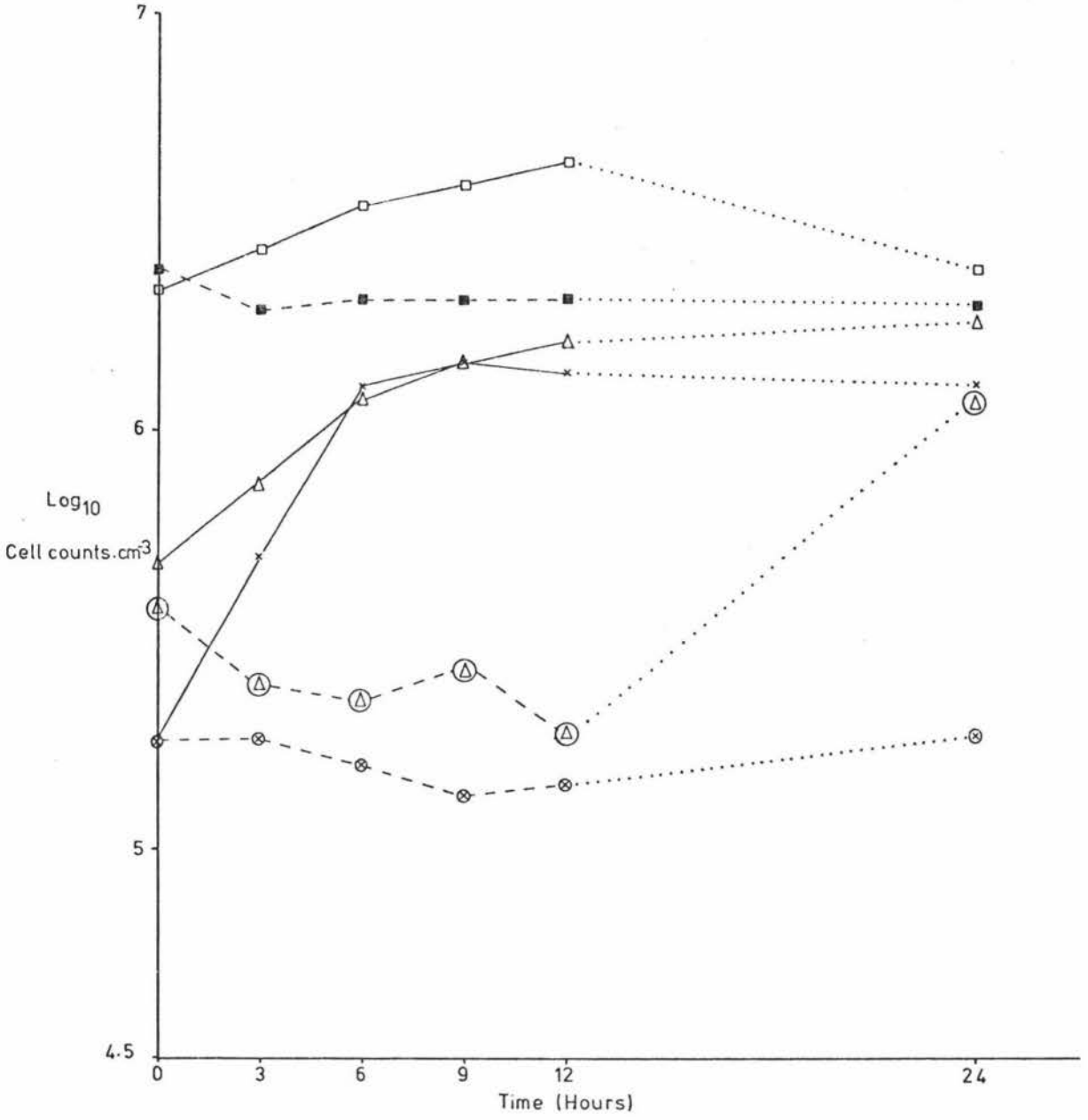


Figure 5-1

Growth of total coliform bacteria in effluent samples held at room temperature and 4C

Key:

- Sewage effluent - room temp.
- Sewage effluent - 4C
- Dairy shed effluent - room temp.
- Dairy shed effluent - 4C
- Freezing works effluent - room temp.
- Freezing works effluent - 4C

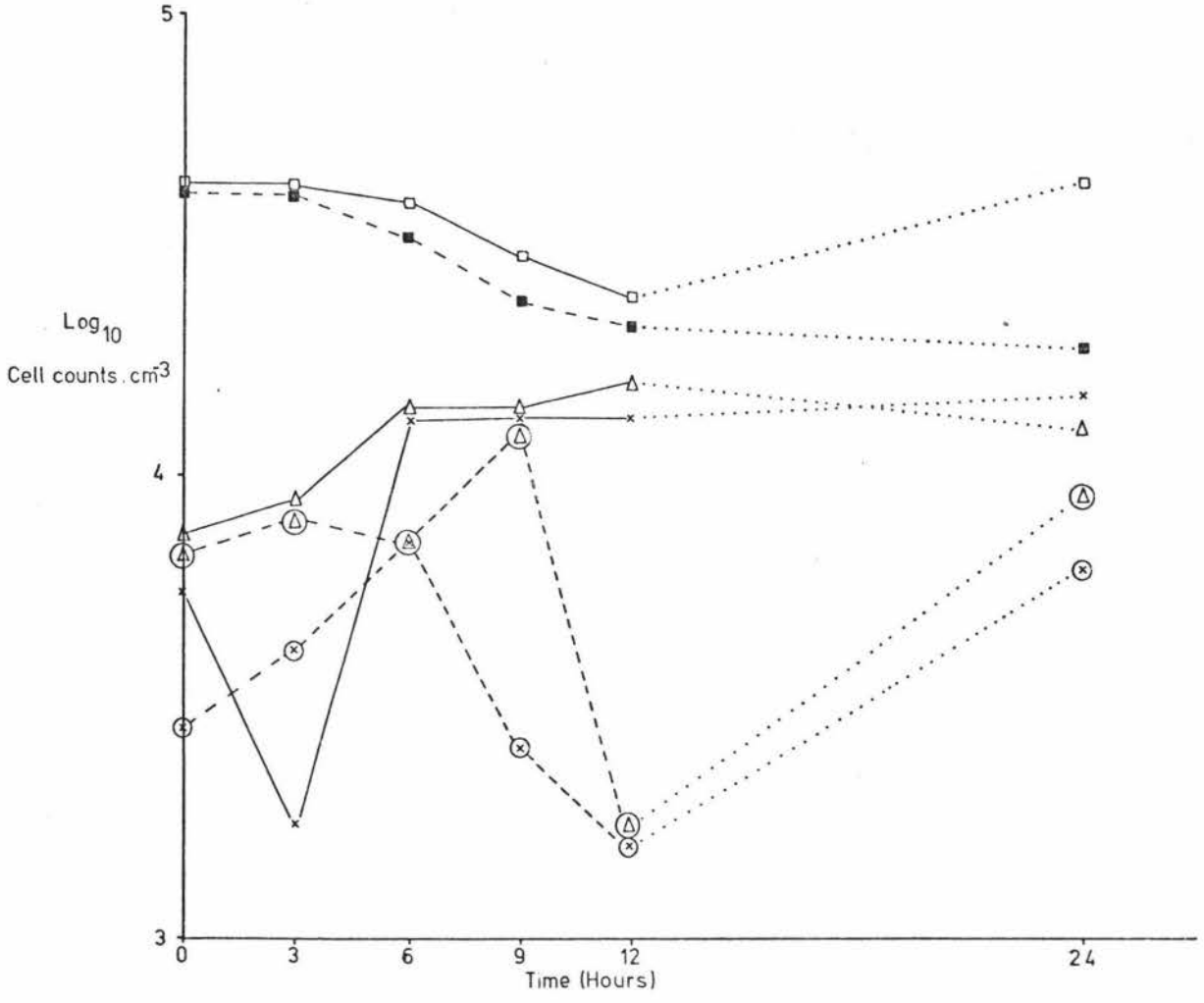


Figure 5-2

Growth of faecal coliform bacteria in effluent samples held at room temperature and 4 C

Key:

- Sewage effluent - room temp.
- Sewage effluent - 4 C
- Dairy shed effluent - room temp.
- Dairy shed effluent - 4 C
- Freezing works effluent - room temp.
- Freezing works effluent - 4 C

the cells or their history prior to storage may well have been responsible. The use of sterilised effluents and pure cultures may have given more information in this regard.

As with total coliforms, 4 C storage resulted in a net decrease in faecal coliforms in 12 hours of storage. Those in freezing works effluent followed an identical trend to the room stored sample. Faecal coliforms in sewage and dairy shed effluents increased initially but then declined rapidly between 6 and 12 hours of storage.

Faecal streptococci populations stayed relatively constant at either storage temperature in both freezing works and sewage effluent (Figure 5-3). In dairy shed effluent incubated at room temperature, however, faecal streptococci increased rapidly after 3 hours of incubation. The rapidity of this increase is reflected in the short mean doubling time (Table 5-1). Storage at 4 C slowed this increase in population but did not halt it completely.

Much has been made of the use of the faecal coliform: faecal streptococci ratio as a means for determining the origin of particular faecal contamination (see Chapter 6). However, the results of this experiment emphasize that care must be taken with the use of such a ratio as it clearly changes with time in some effluent samples (Figure 5-4). For example, in 12 hours there was a 7-fold change in the ratio (0.71-5.2) in room stored sewage effluent and a 10-fold change (2.3-23) in dairy shed effluent at the same temperature.

The reason for the change in ratio differs between effluents. In sewage effluent the dip in the ratio from 0-3 hours was a reflection of a decline in faecal coliform numbers (Figure 5-2). However, the 10-fold decrease in the ratio in room-stored dairy shed effluent was due to the rapid increase in faecal streptococci numbers during this time (Figure 5-3), though faecal coliforms also increased slightly (Figure 5-2).

Storage at 4 C appeared to stabilise the FC:FS ratio for approximately 9 hours in all effluents.

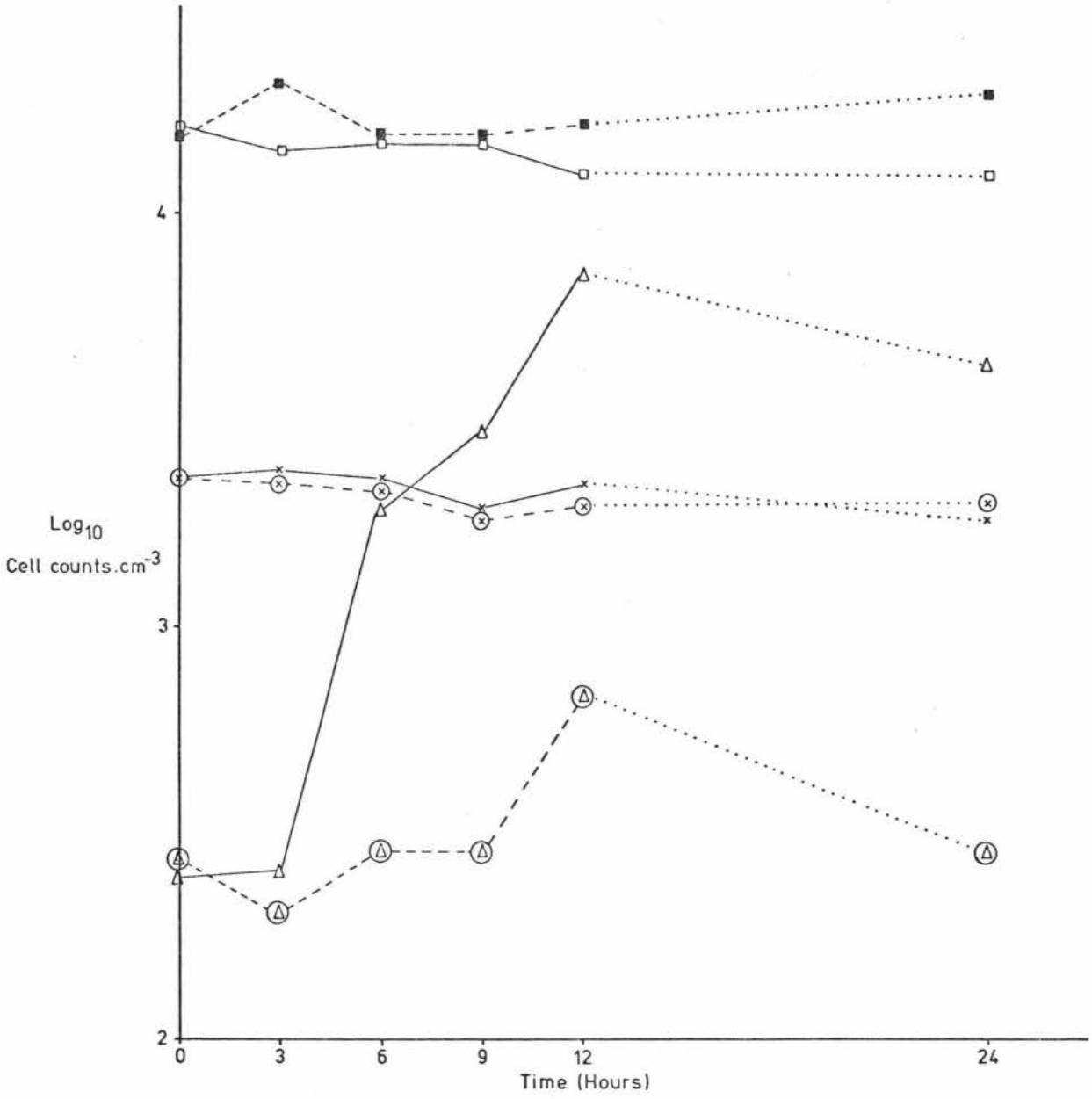


Figure 5 - 3

Growth of faecal streptococci in effluent samples held at room temperature and 4C

Key:

- Sewage effluent - room temp.
- Sewage effluent - 4 C
- Dairy shed effluent - room temp.
- Dairy shed effluent - 4 C
- Freezing works effluent - room temp.
- Freezing works effluent - 4C

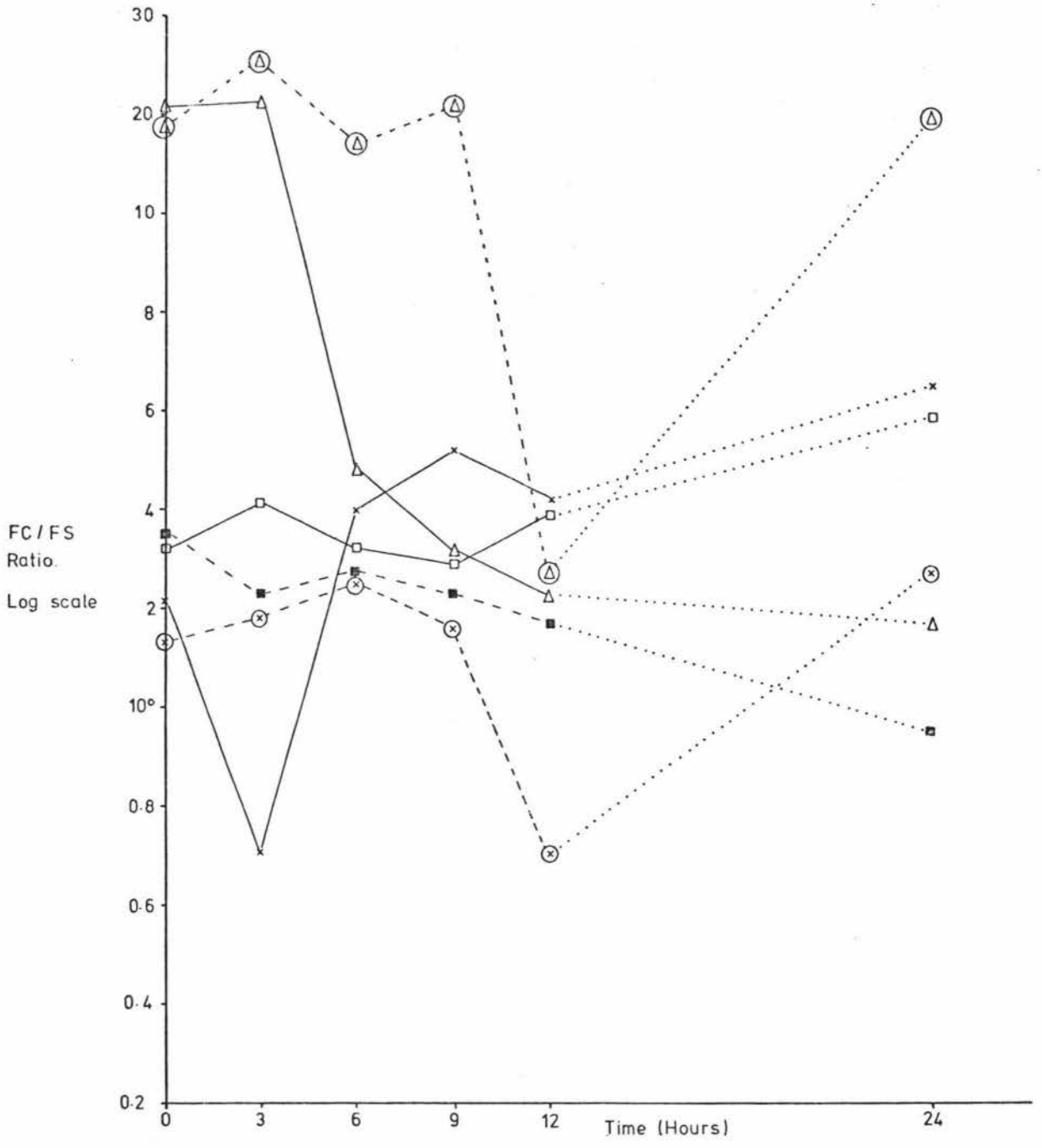


Figure 5-4

Faecal coliform / faecal streptococci ratio in effluent samples held at room temperature and 4C

Key:

- Sewage effluent - room temp.
- Sewage effluent - 4 C
- Dairy shed effluent - room temp.
- Dairy shed effluent - 4 C
- Freezing works effluent - room temp.
- Freezing works effluent - 4 C

5.5 General Discussion and Recommendations

The relative growth patterns of faecal coliform bacteria and faecal streptococci in sewage and dairy shed effluents support the conclusion that faecal coliforms found conditions more favourable for growth in sewage effluent samples (primarily of human origin) than faecal streptococci. The opposite trend is apparent in dairy shed effluent (solely of bovine origin) with faecal streptococci dominating over faecal coliforms. No similar conclusions can be made about the freezing works effluent sample as only total coliforms at room temperature showed any increase in numbers. This may be due to several factors. First, in this effluent, all the bacterial species under consideration had higher initial densities than was the case for the other two effluents. The higher the initial density of bacteria the greater the competition and the greater the chance of a net decrease (Lonsane *et al.*, 1967). Secondly, although freezing works effluent is composed solely of wastes brought about by the slaughter of animals, it would contain a high proportion of bacteria and protozoa of non-faecal origin. Meat processing wastes may contain paunch manure, blood, offal, flesh and fat particles and thus competition and/or antagonism from non-coliform and streptococcus groups would be high. Thirdly, the high pH of the effluent as well as chemical additions from operations such as fellmongery would make conditions less favourable for growth.

The rapid growth rate of faecal streptococci in dairy shed effluent is surprising in view of the findings that:

(i) *Streptococcus bovis* and *S. equinus* are the predominant species of enterococci in cattle (Medrek and Barnes, 1962), and have been reported to compose approximately 70% and 80% of the faecal streptococcus population of bovine faecal material (Geldreich, 1976) and dairy shed effluent (Guy and Small, 1977), respectively.

(ii) *S. bovis* and *S. equinus* die off the most rapidly of all faecal streptococci outside the intestinal tract of the animal (Geldreich *et al.*, 1968; Geldreich and Kenner, 1969; McFeters *et al.*, 1974), and

(iii) M-enterococcus agar (used in this study) is non-selective for the above subgroups (see section 6).

These reports could lead one to conclude that a relatively minor

subgroup of the faecal streptococci in dairy shed effluent was responsible for the recorded rapid growth. This conclusion must be treated with caution, however, as Guy and Small (1977) reported that levels of *S. bovis* isolates, in samples from the same dairy shed effluent discharge used in the present study, were still being maintained or had increased after 24 hours of storage.

Recommendations

Although generalisations are to be treated with caution, the results of this experiment indicate that samples of the effluents under consideration may be held for up to 6-9 hours at 4 C, before significant changes in their indicator bacterial composition become apparent.

CHAPTER 6 CHARACTERISATION OF EFFLUENT DISCHARGES

6.1 Introduction

Rational regional water pollution control planning requires detailed inventories of the composition and volume of all major pollutants from all significant sources. Detailed inventories of even something as fundamental as municipal sewage, however, are few and far between (ACS, 1969).

As New Zealand's economy is based largely on agriculture it is not surprising that a large proportion of its wastewaters are a result of agriculturally-based industries. The dairy and meat industries are chief amongst these. If RWBs are effectively to monitor and issue discharge rights for the wastes generated by these industries then some guidelines as to the most meaningful parameters to monitor are desirable. Background information on the amounts of N and P forms discharged by these industries and municipal sewage is also useful information both for possible nutrient control and for eutrophication prediction.

6.2 Review of literature

6.2.1 Sewage effluent

1. Effluent sources

Municipal sewage is derived from 3 main sources: domestic, stormwater, and industrial. Domestic sewage consists of discharges of spent water from bathrooms and laundries (soapy and dirty water), kitchens (food scraps and dirty water), and lavatories (urine, faeces, paper). It is a complex mixture of mineral and organic matter in many forms, including: (a) large and small particles of solid matter, (b) colloidal and pseudo-colloidal dispersions, and (c) true solution (Hunter and Heukelekian, 1965). Stormwater overflow (in combined systems) may also contribute insoluble mineral matter (sand, clay, gravel, etc.) as well as leaves, animal faeces and other street debris. Most towns and cities also contribute a variety of industrial wastewaters to the sewage system, thus adding an almost indeterminate variety of chemical species.

From this assortment of raw materials it can be seen that the chemical composition of sewage effluent will be very complex. Indeed one of the main features of sewage is its variability (Theroux *et al.*, 1943). Because of possible industrial inputs, comparisons between sewage effluents of different towns and cities are difficult. If domestic sewage is the main input, however, then composition is more uniform.

2. Effluent volumes

A necessary pre-requisite of meaningful comparisons of the volumes of municipal sewage discharged by different cities is a knowledge if the sewered population and area, and also the contributions of industries. If, however, this information is not available then some generalised figures are still possible for purely domestic sewage, which may reflect approximately 40% of the water use of a city (Steel, 1960). The volume of waste contributed per person is strongly related to water usage. Mean domestic sewage volume, therefore, varies from country to country (Table 6-1), apparently reflecting the value placed upon water.

Table 6-1 Volume of sewage produced per head of population in 24 hours (source: Imhoff *et al.*, 1971)

Country	Volume ($\ell.head^{-1}.\ day^{-1}$)	
	Range	Mean
Great Britain	140-300	180
Germany	110-270	160
U.S.A.	300-600	360
Australia	160-300	200

In contrast to the above figures, however, Alexander and Stevens (1976) reported a domestic sewage flow of only 75 $\ell.head^{-1}.\ day^{-1}$ in an Irish housing estate; once corrections had been made for infiltration and seepage into sewage pipes.

3. Solids and pH

Although the rate of water consumption may vary considerably between communities, the total amount of waste solids contributed per person is relatively constant. Approximately 90 g of settleable plus suspended solids is contributed per person in 24 hours (Imhoff *et al.*, 1971). The concentration of solids in the final effluent depends on water use and other contributions, as previously discussed, as well as on the degree of treatment. Generally the concentration of solid matter in effluent after primary treatment is 0.1% or less (Bolton and Klein, 1971).

The pH of fresh sewage effluent, which is not subject to a shock industrial loading, is commonly in the range of 6.8-7.8 (Hunter and Heukelekian, 1965; Lee *et al.*, 1975; Quin, 1975). The pH will drop if the sewage putrefies (Imhoff *et al.*, 1971).

4. Bacteriological parameters

The faeces of man and the sewage wastes he creates are a major source of pathogens that are carried in water (Geldreich, 1972a). Monitoring sewage for pathogens was observed by the above author to be an excellent epidemiological tool for determining the diseases which may be prevalent in the community at the time.

The bacterial content of raw sewage and sewage effluent is directly related to the "strength" of the sewage, i.e., the amount of

excreta contained in a constant volume. Coliform bacteria and in particular faecal coliform bacteria are commonly used indicators of faecal contamination (Wolf, 1972). An "average" human excretes 1.7×10^7 coliform bacteria per gram of faeces (McCalla *et al.*, 1970). Multiplication of coliforms in raw sewage usually occurs (Kittrel and Furfari, 1963), and although these bacteria may die-off during treatment (Klock, 1971), numbers of coliform bacteria in sewage effluent are still high (Steel, 1960; McCoy, 1971). The former author reported coliform densities of 3×10^4 cm^{-3} to 2×10^5 cm^{-3} and the latter author reported *Escherichia coli* densities of 1.8×10^3 to 1.0×10^4 cm^{-3}. Correlations of faecal coliform densities with pathogens have been unpredictable (Gallagher and Spino, 1968). Monitoring of *Salmonella* (which is the only pathogen that can be monitored with relative ease) in sewage has shown that salmonallae may be recovered at any faecal coliform density and recovery is approximately proportional to the incidence of salmonellosis in the community (McCoy, 1971).

Faecal coliforms are dominant over the faecal streptococci group in the faeces of man and a FC:FS ratio of 4.4 has been derived for human faecal material and above 4.0 for various domestic sewages (Geldreich *et al.*, 1968). Ratios from 4.0 to 0.7 have not been adequately classified (Van Donsel and Geldreich, 1971) and may be described as indeterminate as to source. Ratios of FC:FS of less than 0.7 indicate faecal pollution by warm-blooded animals other than man (Geldreich and Kenner, 1969). The possibility of sewage contamination of waters therefore decreases as the FC:FS ratio in those waters decreases.

5. Organics and oxygen-demand parameters

Determinations of sewage composition are usually done for a specific purpose such as checking on the efficiency of a treatment process. Because one of the main effects of sewage effluent on a receiving water is to utilise its capacity for degrading organic wastes, much of the work on sewage characterisation has centred around the identification of organic compounds. Most of the organic matter in sewage is in the particulate fraction (Hunter and Heukelekian, 1965) although the organic acids and sugars are predominantly in the dissolved form. Comparisons between studies (Painter and Viney, 1959; Hunter and Heukelekian, 1965) reveal that although there is little

agreement on the relative quantities of specific groups some generalisations can be made. Carbohydrates plus lignin appear to be the largest constituent group of sewage organic constituents, with grease (largely esterified fatty acids) and amino acids present in smaller but approximately equal amounts. The objectionable odour of sewage is due mainly to the putrefaction of P- and S-containing organic matter by anaerobic bacteria with formation of hydrogen sulphide, organic sulphides, and mercaptans (Bolton and Klein, 1971). Organic amines, especially indole and skatole, impart a characteristic unpleasant faecal odour to sewage (Hunter and Heukelekian, 1965).

In a more recent study, Manka *et al.* (1974) attempted to characterise the organics in secondary sewage effluents. They isolated proteins, carbohydrates, tannins, lignins, anionic detergents, ether extractables, fulvic and humic acids, and hymathomelanic acid. They also identified a number of other organic compounds, such as alkyl benzenes and higher aromatics, which were probably associated with industrial inputs.

The degradation of these organic compounds by organotropic microorganisms requires the use of oxygen to support respiratory activities. This oxygen utilisation may be measured in terms of biological oxygen demand (BOD) and is commonly used as a measure of the "pollutional strength" of the waste (Gaudy, 1972). Steel (1960) defined sewage "strength" in terms of BOD with weak, medium, and strong sewage effluents having BOD_5 levels of 95, 210, and 400 $mg.\ell^{-1}$ respectively. The chemical oxygen demand (COD) of municipal sewage effluent is generally 1.5-2.5 times that of the BOD_5 (Hunter and Heukelekian, 1965), although with weak sewage effluents BOD_5 and COD may be almost identical. These authors fractionated sewage into settleable, supracolloidal, colloidal, and dissolved solids and reported that these fractions contributed 34-40, 23-27, 14, and 23-25 per cent respectively of the total strength of the sewage measured in terms of COD.

6. Trace elements

The inorganic constituents of sewage effluent may include all the ionic species found in the water supply, as well as a variety of chemical species introduced by industry and organic matter decomposition during the treatment process. A knowledge of the trace element composition of sewage is important for several reasons. First, toxic

amounts of metals may accumulate in soils which have been irrigated with sewage effluent (Ward, 1975; Quin and Syers, 1977) or sludge (Berrow and Webber, 1972). Secondly, discharge of sewage effluent containing toxic amounts of trace elements into natural waters may adversely affect aquatic organisms. Thirdly, certain trace elements must be present in sewage effluent in sufficient concentration to enable microorganisms to thrive in a biological treatment system such as activated sludge (Wood and Tchobanoglous, 1975). Some concentrations of trace elements, as reported in the literature, are presented in Table 6-2. The cause of variation between different studies may be due to analytical precision or variations in industrial effluent, treatment type and efficiency, and water supply. The origins of the trace elements appearing in sewage have been studied by several authors. Berrow and Webber (1972) speculated that high levels of Cr in sewage were associated with leather manufacture and that high levels of Zn may have been caused by dissolution of Zn from galvanised metal by rainwater. The amount of Pb in a combined sewer/stormwater overflow system was correlated with an area of paved surfaces by Bryan (1974). Initial high concentrations of Hg in the sewage of a university campus were found to be due to careless laboratory handling (Cox *et al.*, 1975). Informing users on campus that a survey was under way was sufficient to halve Hg concentrations by the end of the study.

Although information on total amounts of trace elements in sewage effluent and sludges is becoming available, few data are available on the chemical forms of the elements. Chen *et al.* (1974) attempted to gain some information on this problem by separating trace elements on the basis of their passage through membrane filters. They reported that most of the Cd, Cr, Cu, Hg, Zn, and Fe in primary effluent was associated with particulates and that Ni, Pb, and Mn were mainly in the dissolved state.

7. Nitrogen and phosphorus forms

The forms and amounts of N and P in wastewaters discharging into natural waters has received a great deal of attention (Vollenweider, 1968), due to the well-established relationships of both these nutrients with accelerated eutrophication (Keeney, 1973; Lee, 1973). As municipal sewage is a major input of nutrients into natural waters (Vollenweider, 1968), and is a point source for which some form of treatment facility usually exists for public health reasons (Bolton and

Table 6-2 Range of trace element concentrations reported in some municipal sewage effluents

Reference	Element																
	Ba	Bi	B	Cd	Co	Cu	Cr	Fe	Pb	Mn	Hg	Mo	Ni	Ag	V	Zn
	mg.ℓ^{-1}										μg.ℓ^{-1}		mg.ℓ^{-1}			
Bryan (1974)									<0.10 – 12.6							
Bradford *et al.* (1975)	0.05 – 0.13		0.3 – 2.5	0.005 – 0.22	0.001 – 0.01	0.006 – 0.053	0.001 – 0.01						0.003 – 0.35		0.001 – 0.009	0.004 – 0.350
Cox *et al.* (1975)											0.25 – 7.20					
Lee *et al.* (1975)		0 – 0.110		0 – 0.020		0.073 – 0.432	0.100 – 1.570	1.29 – 4.01	0.038 – 0.241	0.059 – 0.153	0.60 – 3.3		0.026 – 0.132	0.001 – 0.123		0.243 – 1.473
Wood and Tchobanoglous (1975)					0	0.01 – 0.03		0.01 – 0.12				0			0	0.01 – 0.02
Quin and Syers (1977)				<0.0001	<0.001	0.001	<0.001		0.001	0.015			<0.001			0.015

sewage effluents is difficult, some generalisations on P and N loadings as related to population can be made (Jenkins *et al.*, 1973). From a compilation of 16 sources, Vollenweider (1968) reported a range of 0.65-4.8 g.capita^{-1}. day^{-1} for P and 5.1-15.3 g.capita^{-1}. day^{-1} for N, with means of 2.18 g and 10.8 g respectively. Using purely domestic sewage from a housing estate, Alexander and Stephens (1976) reported a per capita P loading of 1.8 g.day^{-1}. The total P was divided into; 51% DIP (urine and hydrolysis products of polyphosphates), 37% DOP (organic compounds and polyphosphates), and 12% PTP.

6.2.2 Dairy and beef cattle effluent

1. Effluent sources

Historically, far more effort has been devoted to the control of wastewaters derived from human sources (sewage, industry, and urban runoff) than has been the case with agriculturally-derived sources (Middlebrooks, 1974). This has principally been due to public health concern with sewage effluent, and also to the belief that agricultural wastes (especially animal wastes) are a relatively unimportant source of pollution. In the United States, the intensification of agriculture, and the increased public awareness of the degredation of the environment caused by agricultural waste disposal practices,has resulted in recent legislation which makes specific attempts to solve agricultural pollution problems.

In New Zealand the almost total predominance of grazing over housed animals eliminates many of the problems brought about by feedlots and similar enclosures. Intensive dairying practices, however, have brought with them animal waste problems of similar magnitude. Unfortunately there is little information available on dairy-shed effluent and for the purposes of this review, literature pertaining to beef and dairy cattle feedlots has been included.

The manure produced by dairy and beef cattle is very similar (Azevedo and Stout, 1974; Loehr, 1974) and therefore one would expect the effluents resulting from feedlot and dairying operations to be also similar. While this is largely true, several differences, both qualitative and quantitative, should be considered. Qualitative differences may be due to:

(i) variation in feed efficiency between grain and other formulations used in feedlots and pasture grazed by dairy

herds (Taiganides and Hazen, 1966; Azevedo and Stout, 1974),

(ii) inclusion of a small but as yet unmeasured amount of detergent and milk from cup and tank washings in dairy-shed effluent.

Both of these differences are likely to be minor, with the former possibly affecting oxygen-demand parameters and the latter possibly the microbial population (addition of bacteriocides in teat washes, etc.).

Quantitative differences are likely to exist between feedlot and dairy-shed effluents due to the nature of the operations generating them. Whereas cattle spend 24 hours per day in a feedlot, cows are only in the milking shed for 1-3 hours per day. Cleaning operations are more frequent in dairy sheds and the concentration of effluent in dairy-shed washdown is relatively constant (Loehr, 1974; Macgregor *et al.*, 1975), being related to the size of the herd milked and also water use. Feedlot effluent, on the other hand, mainly arises from runoff rather than cleaning operations and its concentration is likely to be highly variable (McCalla and Viets, 1969; McCalla *et al.*, 1972) being dependent on such factors as the intensity of manure accumulation prior to rainfall and also on the volume and intensity of the rainfall.

Thus although differences do exist between soil-filtered dairy shed effluent and feedlot effluent, they both consist of cattle manure modified only by dilution and/or filtration by soil. This is in contrast with anaerobic/aerobic pond systems which drastically change the characteristics of the effluent, and hence are not considered in this review.

2. Effluent volumes

The magnitude of the potential problem which dairy sheds may cause in New Zealand may be gauged by the following figures derived from Hills (1973). The total farm animal population in New Zealand at that time was 73,800,000 producing approximately 450,970,000 kg of manure per day. Of the total animal population dairy cattle in milk numbered only 2,000,000 or 2.7%, however their excreta amounted to approximately 110,000,000 $kg.day^{-1}$ or 24% of the total. Estimates of the actual amount of excreta which falls in the milking yard are very difficult to arrive at. Syers (1974) cited a figure of 5%. Although not making a quantitative estimate, Azevedo and Stout (1974) imply that it could be greater than the above figure. They commented that cows excrete more in

Klein, 1971; McCoy, 1971), it is one source of nutrients which is amenable to control.

The sources of N and P in municipal sewage may vary. Whereas the N in sewage is derived almost solely from excrement (Daniels and Parker, 1973), a significant amount of P may be derived from industrial and domestic sources. These may contribute P as soluble orthophosphate or as complex inorganic phosphates (Nesbitt, 1973). This latter form consists mainly of pyro- and tripoly-phosphates which are used as builders in detergent formulations (Duthie, 1972). The use of detergents has been variously estimated to contribute 30-70% of the total P present in an average U.S. municipal wastewater (Daniels and Parker, 1973). In Californian sewage, Jenkins *et al.* (1973) estimated a figure of 20-40%. No figures are available on the proportion of detergent P in New Zealand municipal wastewaters, however the overall contribution from polyphosphates in detergents is reported to be relatively low (*Soil and Water*, 1977).

Possibly because of their uniform origin, the concentrations of N forms in sewage effluent are relatively constant, despite large variations in geographical location. The concentrations of NH_4-N, NO_3-N, and organic-N as reported in the literature (Besik, 1975; Lee *et al.*, 1975; Quin, 1976) are in the range 10-30, 0.1-2.0, and 0.2-20 $mg.\ell^{-1}$ respectively. The organic-N component shows the most variation in reported concentrations and this is probably due to variation in the degree of treatment prior to sampling. Concentrations of P forms in sewage effluent are more variable both because of variability in origin and also variability in degree of treatment. Because P is more easily removed from sewage than N (Daniels and Parker, 1973), both in primary, secondary, and tertiary treatment systems, inter-effluent comparisons are likely to be meaningless. The importance of P removal from point sources such as sewage for effective eutrophication control (Vollenweider, 1968), has led to studies of the distribution of P in various size fractions in sewage (Huang and Hwang, 1973). These authors reported that the percent distribution in the > 3 μm, 0.025-3 μm, and < 0.025 μm fractions ranged from 29-40, 5-7, and 53-66 respectively for TP; 19-29, 0-8, and 68-81 for inorganic P; and 56-72, 7-17, and 21-27 for organic P.

Although comparisons of P and N concentrations between different

situations where they are excited, such as dairy-shed holding yards. If a cow spends an average 1½ hours, or 6.25% of its day (2 milkings) in a dairy shed, then the proportion of excrement falling in the dairy shed is likely to be nearer 10%.

It would appear that, in general, dairy cattle excrete more than do beef cattle (Table 6-3). This is related to physiological requirements as lactating animals require more feed than dry animals.

Table 6-3 Quantities of manure produced by dairy and beef cattle in one day

Reference	Animal	Wet manure.day^{-1} (kg)	Dry matter (kg)	% of fresh weight
Taiganides & Hazen (1966)	450kg cattle[a]	29.0		17.6
McCalla & Viets (1969)	408kg steer	27.2	4.1	15
McCalla *et al.* (1970)	Cow	23.6		
O'Callaghan *et al.* (1973)	200kg cattle	10.5		11.1
	450kg cattle	26.0		11.1
	Cow	32.0		11.1
Azevedo & Stout (1974)	Beef cattle[b]	24.6	3.6	18.7
	Dairy cattle[c]	46.5	5.8	12.7

a - mean of 4 references.
b - mean of 6 references.
c - mean of 6 references.

Some attempts have been made to correlate manure produced per unit of production. With respect to milk production, Dale and Day (1967) reported a figure of 2.54 kg manure.kg^{-1} milk produced. The actual volume of effluent generated in milking one cow varies with the value put on the water supply and the degree of treatment of the milk at the dairy shed itself. United States estimates vary from 13-130 ℓ.cow^{-1}.day^{-1} (Loehr, 1974), while results of studies at Massey University indicated a volume of 90-150 ℓ.cow^{-1}.day^{-1} (Macgregor *et al.*, 1975).

3. Solids and pH

The concentration of solids in feedlot runoff or dairy-shed washdown effluent will depend on the amount of water applied. The ranges of total solids concentrations reported for both feedlot runoff (0.07-3.3%) and dairy-shed effluent (0.08-1.04%) are comparable (McCalla and Viets, 1969; Gilbertson *et al.*, 1970; McCalla *et al.*, 1972;

Loehr, 1974; Macgregor *et al.*, 1975). Suspended solids in dairy-shed effluent generally range from 220-7190 $mg.\ell^{-1}$ with a mean of approximately 2000 $mg.\ell^{-1}$ (Loehr, 1974).

The pH of effluent from either beef or dairy cattle activities is generally slightly alkaline (McCalla and Viets, 1969; Gilbertson *et al.*, 1970; Satterwhite and Gilbertson, 1972; Macgregor *et al.*, 1975). The mean of values reported by the above authors was 8.4 with a range of 6.7-9.4.

4. Bacteriological parameters

The recent upsurge of interest in the disposal of animal wastes caused by intensive agricultural management has also been accompanied by increasing concern for the public and animal health aspects of animal wastes. While unable to isolate pathogens, Geldreich (1972b) expressed concern that runoff from cattle feedlots may pose a threat to recreational uses of natural waters.

Coliforms and faecal coliforms are not usually considered pathogenic but they may cause coliform mastitis and coliform uterine infections in dairy cows and colibacillosis in calves (Azevedo and Stout, 1974). Although the number of coliforms excreted per gram of manure is only approximately 2×10^5 in cows compared with 1.3×10^7 in humans (McCalla *et al.*, 1972), the volume of manure excreted is such that 4 times as many coliforms are excreted per day from a cow than from a human.

The FC:FS ratio in the faeces of cattle has been reported to be < 0.05 (McCalla and Viets, 1969) though the value used to indicate farm animal pollution is < 0.7 (Geldreich *et al.*, 1968). Of the faecal streptococci, *Streptococcus bovis* has been reported to be the dominant species in beef cattle faeces (Medrek and Barnes, 1962) while Geldreich (1976) reported that the *S. bovis* - *S. equinus* subgroups constituted almost 70% of the faecal streptococcus population in cows. Approximately 60% of the faecal streptococci in waters draining the effluent disposal area. No.4 dairy unit, Massey University, were reported to be *S. bovis* (Guy and Small, 1977).

5. Organic and oxygen-demand parameters

Little work has been done to characterise individual organic groups in dairy or beef cattle wastes, although some workers in the field of animal nutrition have attempted to quantify the amounts of fats and

fibre in manure in order to study the relative efficiencies of feeds (Azevedo and Stout, 1974).

The variability in the organic strength of feedlot runoff is well illustrated by the ranges of 500-12,000 $mg.\ell^{-1}$ and 1300-40,000 $mg.\ell^{-1}$ reported for BOD_5 and COD respectively (McCalla and Viets, 1969; Gilbertson *et al.*, 1970; Middlebrooks, 1974; Filip *et al.*, 1975). Despite the differences in concentration recorded in runoff the actual amounts of oxygen-demanding organics excreted by cattle is reasonably constant (Middlebrooks, 1974), being approximately 1.6 and 9.4 $g.g^{-1}$ of animal.day $\times 10^{-3}$ for BOD_5 and COD respectively. The low BOD_5:COD ratio is probably a reflection of the digestive ability of ruminants (Azevedo and Stout, 1974).

The BOD_5 of dairy-shed effluent typically ranges from 200-4330 $mg.\ell^{-1}$ (Loehr, 1974). If a mean volume of 100 $\ell.cow^{-1}.day^{-1}$ is assumed (Loehr, 1974; Macgregor *et al.*, 1975), then the total amount of BOD_5 producing organics voided in the milking shed is of the order 20-433 $g.cow^{-1}.day^{-1}$.

6. Trace elements

Little work has been done on the trace element composition of dairy-shed effluent. According to Azevedo and Stout (1974), the trace element concentrations likely to be found in dairy cattle manure on a dry weight basis are 56, 222, 28, and 83 $\mu g.g^{-1}$ for Mn, Fe, Cu, and Zn, respectively.

7. Nitrogen and phosphorus forms

Both N and P are found in high concentration in drainage from feedlot operations (Jones, 1976) and dairy-shed washdown (Loehr, 1974). The high concentration of P is almost exclusively due to faecal material (97.3%)while N is more evenly accounted for by faeces (47.6%) and urine (52.4%)(Azevedo and Stout, 1974). Ammonium-N will usually make up the majority of inorganic-N due to rapid hydrolysis of urea (Jones, 1976).

Although the concentrations of N and P in cattle manures are relatively constant (Taiganides and Hazen, 1966; Miner and Willrich, 1970), ranging from 1.5-4.0% and 0.5-1.0%, respectively, the concentrations of N and P forms in dairy-shed effluent can vary markedly, apparently reflecting differences in herd size and water usage. Concentrations of 6-183, 5-625, 0.3-6.5 and 100-4200 $mg.\ell^{-1}$ for

TDP, NH_4-N, NO_3-N, and TKN, respectively, have been quoted for 24 U.S. dairy sheds (Loehr, 1974) and 1.7-6.9, 5.6-8.9, 0.6-8.5 and 110-324 $mg.\ell^{-1}$ for the same four nutrients from one New Zealand dairy shed (Macgregor *et al.*, 1975).

6.2.3 Freezing works effluent

1. Effluent sources

The term 'freezing works' appears to be peculiar to New Zealand. In overseas literature similar plants are called either 'slaughterhouses' or 'meat-packing' plants, depending on the nature of the operation performed. In New Zealand, most meat processing involves export of 'whole animal' carcasses; hence the term freezing works. Some packing is done for the domestic market and also for specialty products for export. As well as these functions a New Zealand freezing works commonly has a fellmongery plant incorporated into it. The characteristics of wastes generated by slaughterhouses and meat-packing processes are very similar (Koziorowski and Kucharski, 1972) except that slaughterhouse wastes are much more concentrated.

The characteristics of freezing-works effluent will vary according to: the type and number of by-product recovery processes (Loehr, 1974; Tarquin, 1974); the type of effluent treatment process employed (Bond and Straub, 1974); and the numbers and types of animals processed (Koziorowski and Kucharski, 1972).

Wastes from a freezing works are diverse in origin. Excreta from animals penned prior to slaughter will contribute a small but significant amount of organic waste (Hoover and Jasewicz, 1967). Most of the waste, however, is generated on the killing floor (Tarquin, 1974). These wastes containing blood, paunch manure, flesh, grease, hair, hide, urine, and offal, have a characteristic brownish, bloodlike appearance and a repugnant odour due to putrefaction of organic compounds, encouraged by a large albumen content (Koziorowski and Kucharski, 1972). If fellmongery wastes are included in the effluent then a variety of chemicals such as boric acid, lime, and fungicides may also be present (Leather and Shoe Research Association, 1972). Washdown cleaning compounds, salt, and preservatives are also likely additions (Crandall *et al.*, 1971).

The international unit of meat works wastes is the hog (pig) equivalent. The amounts of wastes generated in the slaughter of other

animals (Table 6-4) are compared against the amount generated in the slaughter of 1 hog.

Table 6-4 Hog unit equivalents (source: Bond and Straub, 1974)

Animal slaughtered	Waste equivalent
1 hog (pig)	1 hog unit
1 sheep	1 hog unit
1 lamb*	0.5 hog units
1 calf (< 275 kg)	1 hot unit
1 medium beef (275-410 kg)	3 hog units
1 heavy beef (> 410 kg)	5 hog units

*Not from Bond and Straub. An approximation based on the weight of one half-grown sheep.

2. Effluent volumes

The quantity of wastewater generated by a particular freezing works is dependent on water usage and byproduct recovery, although the initial amounts of wastes generated per animal are fairly well defined. Approximately 3, 14, 2, and 1 kg of blood are produced from the killing of 1 pig, beef cattle, calf, and sheep, respectively (Crandall *et al.*, 1972). Loehr (1974) estimated that as much as 22 kg of blood, 4.5-22 kg of paunch manure, and up to 19 kg of faecal manure may be generated as wastes during the slaughter of a single beef animal.

Wash-water and additional wastes produced during processing increase the overall volume of effluent tremendously. Estimates of 0.5-1.8 $m^3.animal^{-1}$ are typical for slaughterhouse operations (Rudolfs, 1953; Koziorowski and Kucharski, 1972; Bond and Straub, 1974), while meat-packing operations are responsible for an even greater volume of wastewater. Estimates of 2.5, 10.5, and 4.7 m^3 for pig, cattle and mixed-packing operations respectively (expressed on a per animal basis) were cited by Bond and Straub (1974). The mean volume of wastes generated by 8 different meat works (Crandall *et al.*, 1972) was 8.2 $m^3.1000\ kg^{-1}$ liveweight.

3. Solids and pH

The pH of effluent from slaughtering or meatpacking operations is generally 7.0-7.3 (Tarquin, 1974), although it may be considerably higher if wastewater from a fellmongery drains into the treatment

plant. The pH of fellmongery effluent is typically 11.5 (Leather and Shoe Research Association, 1972) due to the use of lime for dehairing.

As with other parameters, the solids concentration of both influent and effluent depends on water usage and efficiency of treatment. Concentrations of suspended solids have been reported to vary from 300-12,000 mg.ℓ^{-1} in influents to the treatment plants (Koziorowski and Kucharski, 1972; Loehr, 1974) and from 39-14,000 mg.ℓ^{-1} in effluents, depending on the treatment (Wells and Whitton, 1970; Crandall *et al.*, 1971; Tarquin, 1974). The former authors monitored the wastewaters from two South Island freezing works over a period of 3 years. The mean SS content of influent was 726 mg.ℓ^{-1} and that of the effluent was 39 mg.ℓ^{-1}. The total solids content of fellmongery effluent is typically 10,000 mg.ℓ^{-1} (Leather and Shoe Research Association, 1972).

4. Bacteriological parameters

To this author's knowledge, no work has been published on numbers of indicator bacterial species in freezing works effluents. From the nature of the wastes described, however, it is likely that they will be very high. All the pathogenic microorganisms that are likely to affect farm animals may be found in the effluent (Koziorowski and Kucharski, 1972). In New Zealand, where meat hygiene regulations are strict, animals are vetted both prior to and after slaughter. Diseased animals are rejected but are still slaughtered (Hinde, pers. comm.) and their wastes will be included in the effluent. No estimates are available for the FC:FS ratios likely to be expected in freezing works effluent.

5. Organics and oxygen demand parameters

No quantitative studies of the organic components of freezing works effluent have been published. Any organic compound which occurs in the animal may be present in the effluent, including protein, peptides, amino acids, fats, and grease. Grease (as ether extractables) may be present in concentrations as high as 4450 mg.ℓ^{-1} in effluent (Tarquin, 1974) even after sedimentation and grease skimming.

The primary oxygen-demanding material in freezing works effluent is blood. Blood is an ideal food for bacteria (Crandall *et al.*, 1971) and can have a BOD_5 and COD as high as 156,000 and 218,000 mg.ℓ^{-1} respectively (Loehr, 1974). Paunch material also has a high potential oxygen demand of 50,200 and 177,300 mg.ℓ^{-1} for the same parameters.

Values for BOD_5 can vary from 600-13,000 $mg.\ell^{-1}$ for both influents and effluents (Loehr, 1974; Tarquin, 1974), though typically, values for effluents range from 500-1500 $mg.\ell^{-1}$ (Koziorowski and Kucharski, 1972). COD values are generally 1.5-2.5 times higher than BOD_5 values. On a production basis 8.4-20 kg of BOD_5 are produced for every 1000 kg liveweight slaughtered (Loehr, 1974).

6. Trace elements

The amounts of trace elements in freezing works effluent may be important if the effluent is to be used for irrigation (Wells and Whitton, 1970). The mean concentrations of trace elements in the effluents of two South Island freezing works is given in Table 6-5.

Table 6-5 Trace element composition of two South Island freezing works effluents (source: Wells and Whitton, 1970).

	Li	B	T	V	Cr	Mn	Co	Ni	Cu	Zn	Ga	Rb	Sr	Mo	Ba	Pb
	$\mu g.\ell^{-1}$															
Fairfield	2.5	4.2	205	3	4.3	27	0.5	2.7	3.9	4.8	1.2	3.9	49	.17	26	1.3
Islington	1.5	4.6	51	0.9	1.9	12	<.1	1.6	6.1	8.0	0.4	1.5	23	.11	9.5	1.4

7. Nitrogen and phosphorus forms

Wastes from the killing floor have high organic-N concentrations due to the high protein content of blood (Crandall *et al.*, 1971). Approximately 1.7 kg of organic-N is produced per 1000 kg of liveweight slaughtered (Loehr, 1974). According to Crandall *et al.* (1971), the high quantities of P in the wastewater from slaughtering and dressing operations are due to the use of P-based detergents for cleaning operations, and the use of P-containing chemicals for curing, water treatment, and descaling operations. From their survey of 8 U.S. meatworks, Crandall *et al.* (1971) noted that it is common practice to use phosphoric acid for the cleaning and descaling of stainless steel surfaces. With the exception of the above case, no studies of the concentrations of different P fractions are reported in the literature although some figures for TP are available (Table 6-6).

Table 6-6 Concentrations of N and P forms in meatworks wastewaters

Reference	Remarks	NH_4-N	Org-N	NO_3-N	TN	Sol-P	TP
		mg.ℓ^{-1}					
Crandall *et al.* (1971)	8 U.S. effluents	24-120	4.5-20	0.01-4.0		3.2-18.7	7.0-20
Koziorowski and Kucharski (1972)	Polish effluents	52-80			80-150		
Loehr (1974)	Meatpacking waste		22-240				
	Slaughterhouse waste		36-510				
Tarquin (1974)	Effluent				19-800		
Wells and Whitton (1970)	Raw effluent				140-460		9.3-26
	Treated effluent				-		0.1-0.12

6.3 Materials and methods

6.3.1 Sampling

An ISCO model 1392 automatic sampler was used in all sampling exercises.

Two days prior to sampling, the sample bottle base was half filled with water and the base, complete with bottles and distributor plate, was placed in a freezer. With the sample bottles completely encased in ice in this manner, a far more efficient cooling of samples resulted than using crushed ice as recommended by ISCO (1974).

In accordance with the results of the preservative trial (Chapter 4), $HgCl_2$ was added to sample bottles prior to chemical sampling runs of freezing works effluent so that the final concentration was approximately 50 mg $HgCl_2.\ell^{-1}$ of sample. No preservative was added to any sewage sampling runs (see Chapter 4). As microbiological parameters were measured in all dairy shed runs, no preservative was added.

Sampling intervals of 15 minutes, 30 minutes, or 1 hour were selected according to flow conditions.

Not all of the large number of analytical parameters involved could be effectively monitored in any one sampling run. The analytical parameters monitored in any one sampling run, the duration of sampling, and the number of samples taken are given in Table 6-7. Because of the range of analytical parameters used, an order of priority of analyses was devised (Table 6-8). This order was based on the results of the preservation experiment (Chapter 4), the bacterial growth experiment (Chapter 5), and on previous analytical experience.

6.3.2 Computation

On completion of all analyses the data were collated and transferred to computer cards. A computer program was used to compute loads from concentration and flow data, to calculate the derived analytical parameters (DOP, PTP, TN, TDN, TPN, as SS), and to find maximum and minimum values in terms of both concentration and load. Mean concentration values reported were flow-weighted.

Statistical analysis of data was done using various routines available on SPSS (Statistical Package for the Social Sciences).

All computing was done on a Burroughs B6700 computer.

Table 6-7 Description, duration, analytical parameters, and number of samples taken in each sampling run.

Parameter	Sampling Run[a]									
	F1	F2	S1	S2	D1	F3	D2	D3	F4	S3
DIP	*	*		*	*			*		*
TDP	*	*	*	*	*	*		*		*
TP	*	*	*	*	*	*		*		*
NO_3-N		*		*	*			*		*
NH_4-N	*	*		*	*			*		*
TKN	*	*	*	*	*	*		*		*
DKN	*	*	*	*	*	*		*		*
BOD_5	*		*		*		*		*	
COD			*		*		*		*	*
DO		*					*		*	
TS	*	*		*	*	*		*		*
DS		*								
TC			*		*	*		*		
FC			*		*	*		*		
FS			*		*	*		*		
pH	*	*		*	*			*		*
COL		*	*	*	*	*	*	*	*	*
UV		*	*	*	*	*	*	*	*	*
Date of commencement	15/4/75	23/3/76	23/6/76	9/8/76	16/9/76	11/11/76	24/11/76	29/11/76	8/12/76	9/12/76
Duration of sampling (Hr.)	72	72	24	48	24	24	24	24	10	30
Number of samples	65	98	25	60	38	24	34	29	11	30

a - Where F1 = Freezing works effluent sampling run No.1, S1 = Municipal sewage effluent sampling run No.1, D1 = Dairy shed effluent sampling run No.1, etc.

Table 6-8 Order of priority for determination of analytical parameters in a sampling run.

Priority	Parameter	Remarks
1	DO	Measured *in situ*.
	TC, FC, FS	Colonies counted after appropriate period of incubation.
	BOD_5	Final reading taken after 5 days.
	pH	
	DIP	
	filtration for other dissolved constituents.	
2	NH_4-N	
	NO_3-N	
3	COL	
	UV	
4	DKN	
	TKN	
5	TDP	
	COD	
6	TP	
	TS	
	DS	

All samples were stored at 4 C.

6.4 Results and Discussion

6.4.1 Sewage effluent

1. Effluent sources

The Palmerston North city sewage treatment plant currently serves a connected population of approximately 58,000 people. Industrial inputs into the sewage system are minor and almost entirely related to food and drink processing (Table 6-9).

Table 6-9 Major industries discharging into the Palmerston North sewage system.

Name of industry	Activity contributing to waste
Fermentation Industries	Yeast manufacture
New Zealand Breweries	Brewing
Farm Products	Brewery waste processing
Palmerston North Milk Treatment Station	Milk treatment
Prepared Foods Ltd.	Food processing
Massey University D.S.I.R. D.R.I.	Domestic and laboratory wastes

The amounts of wastes contributed by the above sources on a population equivalent basis is not known. Discharge is sporadic, however, and should not markedly influence the load of sewage on a long-term basis. Fermentation Industries is a possible exception, and this discharge may affect the BOD:COD ratio in the short term (see Chapter 7), as the biodegradability of yeast waste is low (Wu and Kao, 1976). Discharge of industrial wastes into the sewage system at night is encouraged (Anderson, pers. comm.) because of the light loading from domestic sources at that time.

2. Effluent volume

The average volume of effluent leaving the treatment plant in 24 hours was 25,645 m^3 for the sampling events monitored. Working on a sewered population of 58,000 people this corresponds to an effluent discharge of 442 $\ell.person^{-1}.day^{-1}$ which is a large volume, even by world standards (see Table 6-1).

The mean flow rate recorded was 0.210 $m^3.sec^{-1}$, with a standard

deviation of 0.104 $m^3.sec^{-1}$, and a range of 0.004-0.472 $m^3.sec^{-1}$.

3. Solids and pH

The pH of sewage effluent was rather constant, having a mean of 6.96, a standard deviation of 0.2, and a range of 6.6-7.5. Although variation in pH was slight, a distinct diurnal variation was noted (see Chapter 7).

The total solids concentration of sewage effluent was highly variable. The mean was 313.5 $mg.\ell^{-1}$ and the standard deviation 205.5 $mg.\ell^{-1}$. For the three sampling days in which solids were analysed, the minimum concentration observed was constant at 40 $mg.\ell^{-1}$; the maximum concentration varied widely. Further discussion of time- and flow-based variations is given in Chapter 7.

The mean TS discharge into the Manawatu River was 117.3 $g.person^{-1}.day^{-1}$, which is about average for those reported in the literature (section 6.2.1). This would indicate that the large volume of effluent discharged per capita per day is due to extravagant water use rather than to a larger than (internationally) normal amount of wastes to dispose of.

4. Bacteriological parameters

The indicator bacterial composition of Palmerston North sewage effluent (Table 6-10) is consistent with that reported for other sewage effluents (section 6.2.1).

Table 6-10 Concentration of indicator bacteria in Palmerston North sewage effluent.

Group	Mean	Standard deviation	Maximum	Minimum
		$n.100\ m\ell^{-1}$		
TC	2.14×10^7	1.91×10^7	6.50×10^7	1.60×10^6
FC	4.58×10^6	3.73×10^6	1.50×10^7	1.00×10^6
FS	6.15×10^5	2.88×10^5	1.40×10^6	1.40×10^5

The number of TC, FC, and FS discharged in 24 hours amount to 4.7×10^{10}, 1.0×10^{10}, and $1.8 \times 10^9.person^{-1}$ respectively. A mean FC:FS ratio of 6.9 indicated that the waste originated dominantly from human faecal material.

5. Oxygen-demand parameters

The mean concentrations of BOD_5 and COD (Table 6-11) indicate that

the majority of oxygen-demanding organics in the effluent are biologically oxidizable in 5 days.

Table 6-11 Concentration of BOD_5 and COD in Palmerston North sewage effluent

	Mean	Standard deviation	Maximum	Minimum	No. of cases
			$mg.\ell^{-1}$		
BOD_5	155	71.2	327	30	20
COD	180	131.2	550	30	55

BOD_5 and COD are strongly correlated (Figure 6-1) with a coefficient of 0.91. BOD_5 may be predicted from COD by the regression equation: $BOD_5 = 0.47\ COD + 45.97$.

Total daily discharges of BOD_5 and COD are fairly constant but may be upset by shock industrial loadings (Chapter 7). The average per capita daily contribution to the effluent discharge is 46.6 and 74.2 g of BOD_5 and COD, respectively.

The DO concentration in the sewage effluent ranged from 0.4-1.6 $mg.\ell^{-1}$ with a mean of 1.0. Due to the use of *in situ* analyses only 10 DO readings were taken and all in daylight hours.

7. Nitrogen and phosphorus forms

The concentration of N and P forms (Table 6-12) were relatively constant. Over 50% of the total of both P (Figure 6-2) and N (Figure 6-3) was present in dissolved inorganic form. The dissolved organic and total particulate fractions accounted for slightly less than, and slightly greater than 20%, respectively.

Mean TP and TN exports in the sewage effluent amounted to 2.02 and 14.73 $g.person^{-1}.day^{-1}$, respectively. Although the TP value is approximately equal to the mean of values reported by Vollenweider (1968), the TN value is at the upper limit of the above author's compiled values (section 6.2.1).

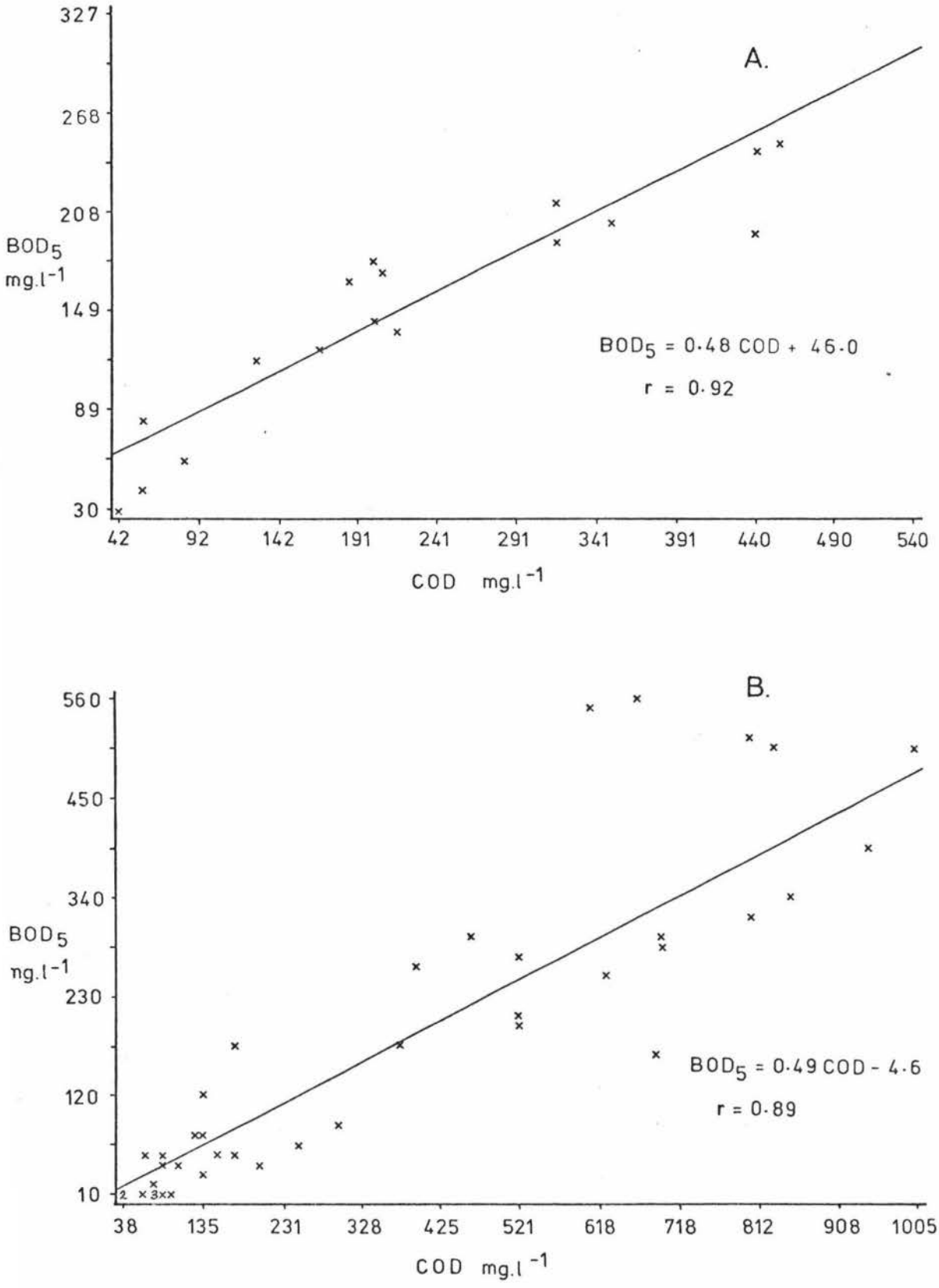


Figure 6 - 1 Relationship between BOD_5 and COD in municipal sewage (A) and dairy shed (B) effluents.

Table 6-12 Concentration of N and P forms in Palmerston North sewage effluent.

Parameter	Mean	Standard deviation	Maximum	Minimum	No. of cases
		$mg.\ell^{-1}$			
TP	4.50	1.85	10.85	1.34	115
TDP	3.27	1.44	8.16	0.83	115
DIP	2.56	1.05	5.24	0.77	90
PTP	1.23	0.98	5.30	0.03	115
DOP	0.70	0.81	3.15	0.01	90
TN	32.0	12.2	69.1	14.0	90
TKN	31.3	11.6	69.0	13.1	115
DKN	27.0	10.3	56.6	7.5	115
NH_4-N	19.2	6.9	43.5	7.0	90
NO_3-N	0.6	0.5	2.6	0.0	90
TDN	27.7	10.9	56.7	8.4	90
TPN	3.6	4.1	19.2	0.0	115

6.4.2 Dairy shed effluent

1. Effluent sources

The primary source of raw effluent was the yard washdown during and after a milking event. Bovine faecal material was the main constituent but the effluent also contained a small amount of milk and detergent from the washing of the milking equipment. The 'treated' effluent collected from the outlet of the tile drainage system during sampling runs 2 and 3 consisted of, essentially, soil-filtered 'raw' effluent. Because the entire disposal area was above field capacity during the first sampling run, however, the effluent was modified by the addition of soil-water. This soil-water would have contained leachate of previously applied effluent, as well as constituents found in agricultural soils characteristic of the area, which have not been treated with effluent (Turner *et al.*, 1976).

2. Effluent volume

The total volume of effluent leaving the disposal area in 24 hours (Table 6-13) varied greatly between seasons.

The approximately 4-fold difference in the volume of effluent between runs 1 and 3 may be attributed to seasonal differences and the 2-fold difference between runs 2 and 3 to variation in water use;

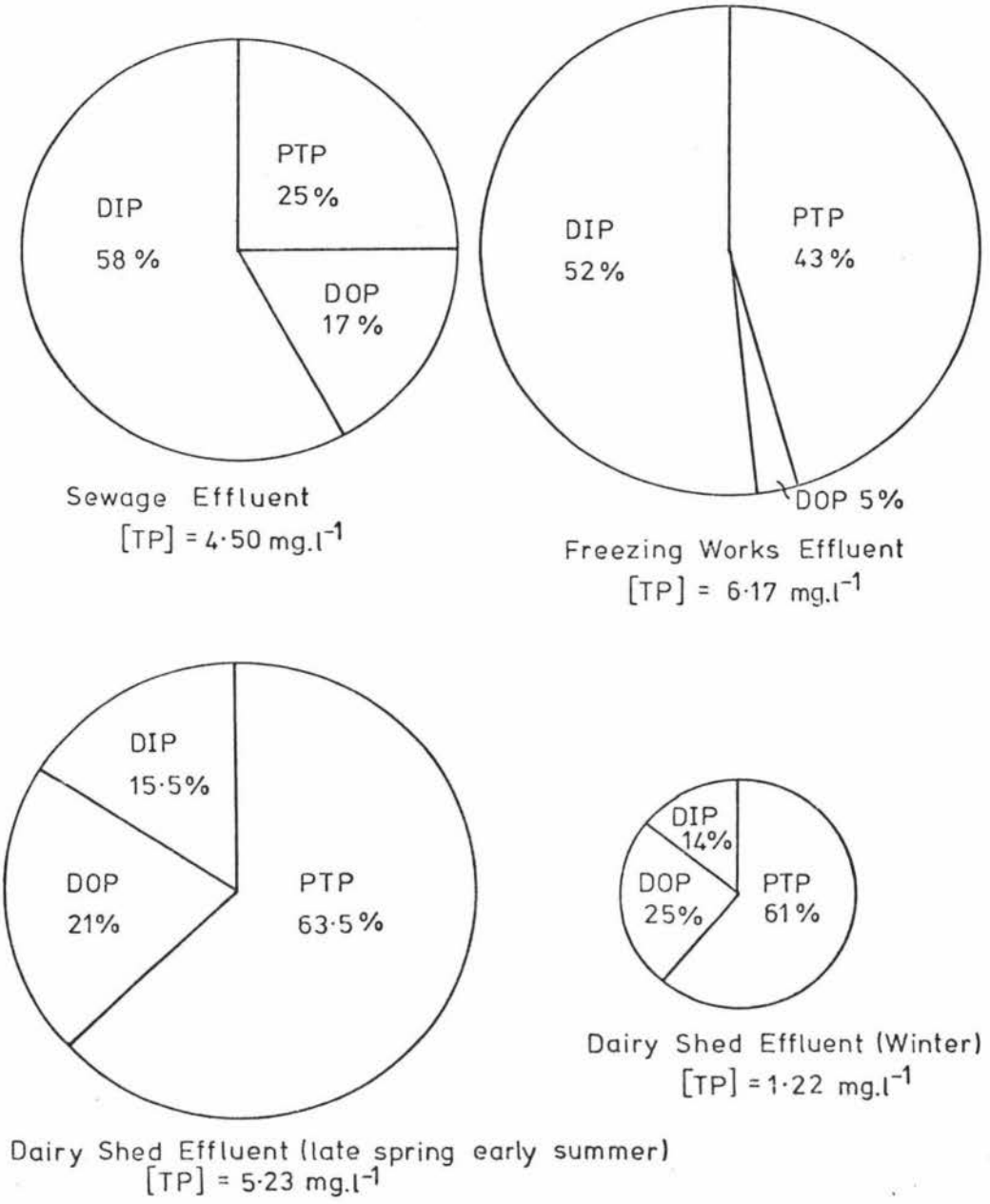


Figure 6-2 Comparison of mean concentrations of total P, and percentages of P forms occurring in effluent discharges

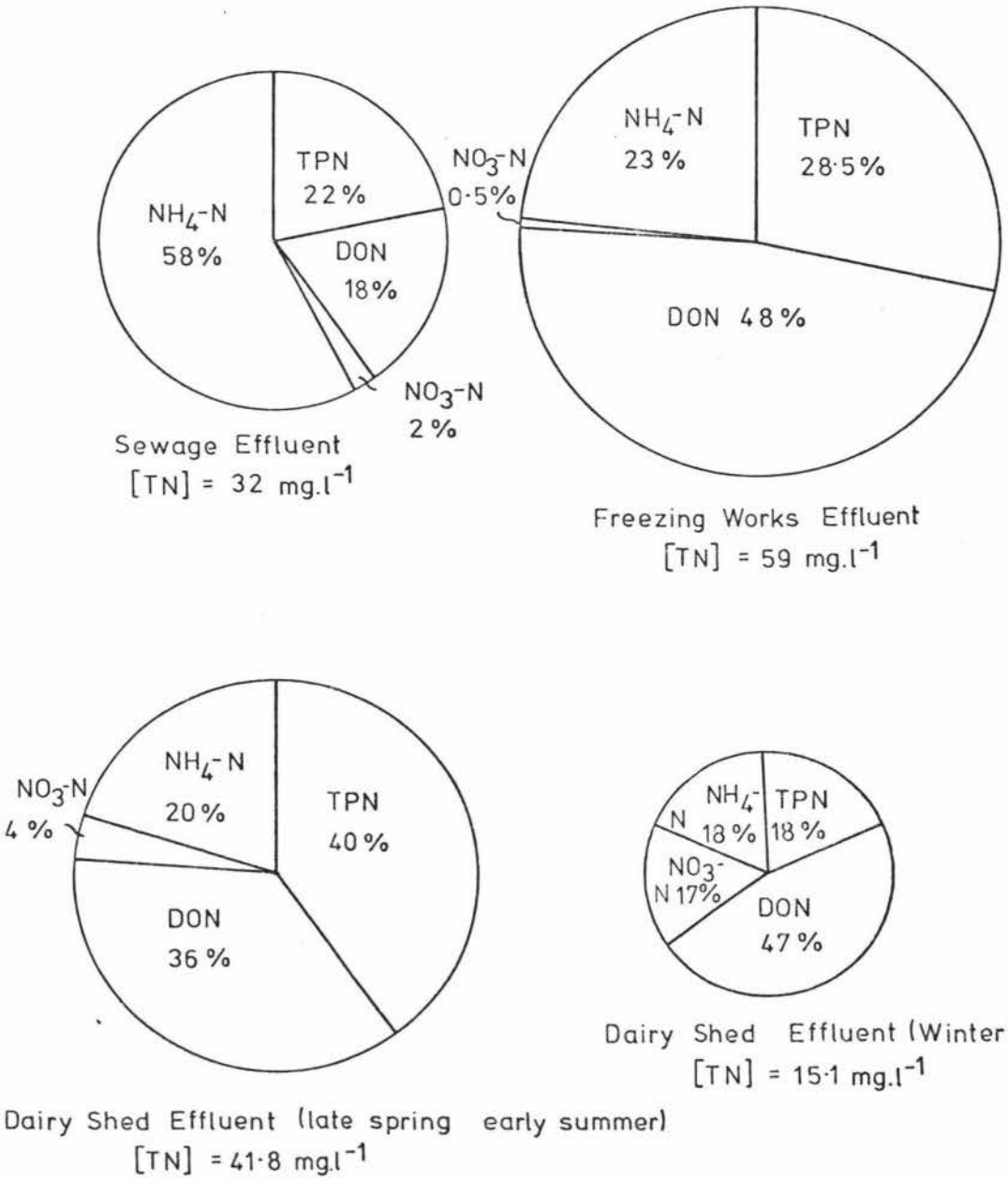


Figure 6-3 Comparison of concentrations of total N, and percentages of N forms occurring in effluent discharges

Table 6-13 Volume of effluent exported from No.4 Dairy Unit effluent disposal area in 24 hours.

Run number	Volume exported m^3	Cow numbers	Volume.cow^{-1} ℓ
1	78.09	230	339.5
2	35.18	300	117.3
3	18.12	300	60.4

though it was suspected (visual evidence), that a blockage in one of the spray units caused a substantial amount of effluent to be poured rather than sprayed on to the site during run 2.

3. Solids and pH

The pH of the effluent for the two runs in which pH was monitored was very constant, with means and standard deviations of 6.83 (0.24 S.D.) and 6.65 (0.15 S.D.) for runs 1 and 3, respectively. Evidently the soil modified the pH of the raw effluent which was between pH 8.0-8.5.

Although the concentration of total solids in the effluent varied widely between runs 1 and 3 (Table 6-14), the total loading of solids in 24 hours was very similar and the loading per cow milked, virtually identical.

Table 6-14 Concentration and load of total solids in dairy shed effluent.

Run no.	Mean	Standard deviation	Maximum	Minimum	Total discharge	Discharge cow^{-1}.day^{-1}
	mg.ℓ^{-1}				g	
1	143	100	400	30	11232	49
2	766	264	1360	90	14332	48

Thus it would appear that rainfall-runoff added only a small amount of solids to the total effluent.

4. Bacteriological parameters

The indicator bacterial composition of dairy shed effluent was highly variable (Table 6-15), both within and between sampling runs. The total discharge of these bacteria, however, was similar for both sampling runs. Total coliforms were the exception, being of a power of 10 higher in the latter sampling run. This was possibly because of a

proliferation of non-faecal coliforms in the soil due to more favourable environmental conditions, or alternatively, to more favourable survival/growth conditions in the effluent itself.

A feature of these results is the low incidence of faecal streptococci. The mean FC:FS for runs 1 and 3 are 27 and 13, respectively, both of which would lead one to classify the effluent as derived from human sources. A full discussion of this apparent anomally, in conjunction with results of the growth/dieoff experiment (Chapter 5), is given in section 6.5.2.

5. Oxygen-demand parameters

In contrast to previously discussed parameters in dairy shed effluent, a large difference in the load of oxygen-demanding organics which could be attributed to each cow occurred between runs 1 and 2 (Table 6-16). This could possibly be related to milk production. At the time the first sampling run was undertaken cows were just coming into milk, whereas by the time the second run took place, cows were near peak production. The feed requirements of cows increase with milk production (Azevedo and Stout, 1974) and so do the concomitant organic faecal residues. Although this may account for some of the increase it seems unlikely that it would account for the greater than 2-fold increase per cow that took place. Another possibility may have been a failure in one of the sprinklers (section 6.4.2.2), leading to saturation of one point of the disposal area and a consequent loss in ability of the system to remove organics and nutrients from the effluent. As only oxygen-demand parameters were measured in the second sampling run, no further check can be made of this hypothesis.

As with sewage effluent, a strong correlation existed between BOD_5 and COD (Figure 6-1), and BOD_5 may be predicted by the equation: $BOD_5 = 0.49\ COD - 4.6$. The high COD:BOD_5 ratio in the effluent can be attributed to the digestive ability of the cow. The efficient nature of its digestive system results in a high proportion of only slowly bio-oxidizable organics, such as lignin (Azevedo and Stout, 1974) in its excreta.

The DO concentration of the effluent, which may be a reflection of instantaneous oxygen demand, was only monitored in the second sampling run. The mean concentration was 2.1 $mg.\ell^{-1}$ with a range of 1.5-2.4 $mg.\ell^{-1}$.

Table 6-15 Concentration and load of indicator bacterial groups in dairy shed effluent.

Parameter	Mean concentration (n.100 $m\ell^{-1} \times 10^4$)		Standard deviation		Maximum		Minimum		Total discharge.day^{-1} n		Total cow^{-1}.day^{-1}	
	Run 1	Run 3	Run 1	Run 3	Run 1	Run 3	Run 1	Run 3	Run 1	Run 3	Run 1	Run 3
TC	80.8	2020	81.6	2130	350	9000	12.0	220	6.47×10^{11}	4.20×10^{12}	2.81×10^{9}	1.40×10^{10}
FC	34.9	110	44.4	123	176	480	0.90	5.5	3.13×10^{11}	2.38×10^{11}	1.36×10^{9}	7.90×10^{8}
FS	1.22	7.42	1.55	6.25	6.50	27	0.16	0.85	1.02×10^{10}	1.68×10^{10}	4.40×10^{7}	5.6×10^{7}

Table 6-16 Concentration and load of BOD_5 and COD in dairy shed effluent.

Parameter	Mean concentration (mg.ℓ^{-1})		Standard deviation		Maximum		Minimum		Total discharge.day^{-1} g		Total cow^{-1}.day^{-1}	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
BOD_5	44	283	44	163	170	560	10	45	3825	8277	16.6	36.0
COD	103	529	49	274	270	1005	38	112	8560	17381	28.5	58.0

6. Nitrogen and phosphorus forms

In both sampling runs, over 60% of the P was in particulate form (Figure 6-2) whereas particulate N varied from 18% winter sampling run to 40% in the early summer run (Figure 6-3). The high proportion of particulate P was probably attributable to the predominance of P in the faeces (c.f. urine) of cattle (section 6.2.2.7).

Although the total discharge of P forms per cow per day was very similar for both sampling runs, large discrepancies existed in the total discharge of N (Table 6-17). While concentrations of N were far higher in the summer sampling run, the actual loading of total N was only 51% that of the winter sampling run. As the difference in loading (Δ) amounted to 2.6, 0.8, 0.4, and 1.8 g for TN, NO_3-N, NH_4-N, and TKN, respectively, and ΔTPN was very small, it is apparent that most of the difference can be attributed to the DON and NO_3-N components.

During the early summer sampling run, almost all of the treated effluent discharging from the weir was due to raw effluent sprayed on to the pasture at discrete sites during the previous milking event. In contrast to this, approximately 50-75% of the winter sampled effluent was due to runoff contributed from the entire disposal area. From the observations (Table 6-17) that the NO_3-N concentrations of the winter effluent samples were;

(i) generally higher than the summer effluent, despite the increased volume due to runoff, and

(ii) more uniform over the entire sampling event (lower standard deviation),

it is evident that most of the NO_3-N in the winter-sampled effluent was contributed from runoff. The additional increment of NO_3-N in the runoff waters was probably due to leaching from the upper soil horizons of NO_3-N which had accumulated during nitrification of both soil organic matter and also effluent applied during the previous milking season.

The lower total loads of DON and NH_4-N in the summer run may partly be due to similar causes. An additional possibility, however, is that in the more favourable environmental conditions (higher temperature, better soil aeration), substantial losses of N could have occurred due to ammonia volatilisation from the pasture surface and the top few

Table 6-17 Concentration and load of N and P forms in dairy shed effluent

Parameter	Mean concentration ($mg.\ell^{-1}$)		Standard deviation concentration ($mg.\ell^{-1}$)		Maximum ($mg.\ell^{-1}$)		Minimum ($mg.\ell^{-1}$)		Total discharge.day^{-1} g		Discharge.$cow^{-1}.day^{-1}$	
	Run 1	Run 3	Run 1	Run 3	Run 1	Run 3	Run 1	Run 3	Run 1	Run 3	Run 1	Run 3
TP	1.22	5.20	0.70	3.10	3.07	12.09	0.38	2.67	94.8	104	0.4	0.3
TDP	0.49	2.00	0.32	0.83	1.18	4.06	0.12	1.18	37.1	38.0	0.2	0.1
DIP	0.16	0.87	0.11	0.36	0.53	1.88	0.05	0.36	13.5	16.1	0.06	0.05
PTP	0.74	3.22	0.61	2.82	2.30	8.93	0.03	0.17	57.3	69.4	0.25	0.2
DOP	0.32	1.14	0.27	0.66	1.02	3.12	0.03	0.14	23.6	21.8	0.1	0.1
TN	15.1	41.8	7.0	23.7	33.4	106.6	5.4	21.4	1202	823	5.3	2.7
TKN	12.6	40.2	7.2	22.9	31.4	102.8	2.8	20.7	1003	791	4.4	2.6
DKN	10.0	23.3	4.8	11.4	21.0	52.3	2.7	13.3	785	463	3.4	1.5
NH_4-N	2.6	8.3	1.6	4.2	7.4	18.9	0.1	2.4	217	163	0.9	0.5
NO_3-N	2.5	1.6	0.3	1.0	3.2	3.8	1.8	0.4	199	32	0.9	0.1
TDN	12.5	24.9	4.6	12.2	23.0	56.1	5.2	14.5	984	495	4.3	1.6
TPN	2.6	16.8	2.8	12.4	11.2	50.5	0.1	5.4	218	328	1.0	1.1

centimetres of the soil. Urea hydrolysis to ammonia has been shown to be rapid in this disposal system (Tan and Macgregor, pers. comm.) and this, as well as the high pH of the raw effluent (section 6.4.2.3), emphasizes the possibility of ammonia volatilisation.

6.4.3 Freezing works effluent

1. Effluent sources

The freezing works effluent at Longburn results from the killing and processing of lambs, sheep, and cattle by the Longburn Freezing Company, and pigs by the Kiwi Bacon Company. The numbers of lambs, sheep, cattle, and pigs slaughtered on each of the sampling dates is given in Table 6-18. As liveweights were not known, the hog unit equivalent (see Table 6-4) for cattle is assumed to be 4.

Table 6-18 Number of lambs, sheep, cattle and pigs slaughtered at Longburn on effluent sampling days.

Date	Lambs	Sheep	Cattle	Pigs	Total (hog equivalents)
	number killed				
15/4/75	4437	363	172	0	3270
16/4/75	5493	7	166	372	3590
17/4/75	5158	64	152	372	3623
18/4/75	3640	160	128	317	2809
23/3/76	4593	535	172	0	3520
24/3/76	4173	988	158	0	3707
25/3/76	4558	46	0	407	2732
26/3/76	3564	649	82	488	3247
11/11/76	3212	37	91	421	2428
12/11/76	0	0	0	289	289
8/12/76	6220	218	125	0	3828

2. Effluent volume

As only discrete flow observations were available (section 3.1.3) the accuracy of computation of the total volume of effluent was not as good as for the other two effluents. Estimates of night flows were particularly suspect in this regard as they were dependent on a continuous 'flushing' pump which was prone to breakdowns. Night flows constituted a relatively minor proportion of the total flow.

Estimates of flow ranged from 3,500 $m^3.day^{-1}$ to 21,900 $m^3.day^{-1}$,

although the higher value was exceptional. In general, flow varied between 3,500-6,500 $m^3.day^{-1}$ with a mean of 4,500. As the number of hog equivalents slaughtered was fairly constant (Table 6-18) a mean effluent output of 1.3 $m^3.hog\ unit^{-1}$ can be calculated, and this is within the range of estimates reported in the literature (section 6.2.3.2).

3. Solids and pH

The pH of the freezing works effluent was generally slightly alkaline having a mean of 7.8 and a range of 7.0-9.5. The variability in pH (standard deviation 1.04 pH units) was probably a function of the fluctuating quantities of highly alkaline fellmongery effluent entering the treatment plant.

The concentrations of the various solids fractions (Table 6-19) were lower than those generally quoted in overseas literature (section 6.2.3), although comparisons are difficult when the degree of treatment at the point of sampling is not known.

4. Bacteriological parameters

The concentration of all the indicator bacterial species (Table 6-20) was higher than for the other effluents, as was total discharge in terms of population units. The mean FC:FS ratio was 5.7 which again could lead one to believe that the effluent was from human origins. Possible reasons for this anomally are given in section 6.5.2.

A feature of the indicator bacterial population in the freezing works effluent was the high incidence of non-faecal coliforms (TC-FC). This emphasizes the possibility of competitive effects (Chapter 5) on faecal indicator bacterial survival, as there was undoubtedly a high population of other non-faecal bacteria and possibly protozoa.

5. Oxygen-demand parameters

Between 75 and 95% of COD organics in the freezing works effluent were biologically oxidizable in 5 days (Table 6-21). The high proportion of COD as BOD_5 was probably due to blood and paunch washings which are an ideal medium for bacterial growth (Crandall *et al.*, 1971).

As with dairy shed effluent, few DO determinations were made and all in daylight hours. The mean of these 6 determinations was 1.8 $mg.\ell^{-1}$ with a standard deviation of 0.4 $mg.\ell^{-1}$. Although it is

Table 6-19 Concentration and load of solids fractions in freezing works effluent.

Parameter	Mean concentration ($mg.\ell^{-1}$)	Standard deviation concentration ($mg.\ell^{-1}$)	Maximum concentration ($mg.\ell^{-1}$)	Minimum concentration ($mg.\ell^{-1}$)	Mean discharge.day^{-1} kg	Discharge.hog^{-1}.day^{-1} kg
TS	1082	347	1740	270	6,617	2.00
SS*	207	275	710	30	1,606	0.49
DS*	863	103	1310	270	5,011	1.51

*One sampling run only.

Table 6-20 Concentration and load of indicator bacterial groups in freezing works effluent.

Parameter	Mean concentration (n.100 $m\ell^{-1}$)	Standard deviation concentration (n.100 $m\ell^{-1}$)	Maximum concentration (n.100 $m\ell^{-1}$)	Minimum concentration (n.100 $m\ell^{-1}$)	Total discharge.day^{-1} n	Discharge.hog^{-1}.day^{-1} n
TC	2.08×10^{8}	1.70×10^{8}	6.60×10^{8}	4.10×10^{7}	4.00×10^{15}	1.6×10^{12}
FC	3.50×10^{6}	1.48×10^{6}	6.70×10^{6}	1.90×10^{6}	1.57×10^{14}	6.3×10^{10}
FS	6.40×10^{5}	2.43×10^{5}	1.26×10^{6}	2.45×10^{5}	2.58×10^{13}	1.0×10^{10}

Table 6-21 Concentration and load of BOD_5 and COD in freezing works effluent.

Parameter	Mean concentration ($mg.\ell^{-1}$)	Standard deviation concentration ($mg.\ell^{-1}$)	Maximum concentration ($mg.\ell^{-1}$)	Minimum concentration ($mg.\ell^{-1}$)	Mean discharge.day^{-1} kg	Discharge hog^{-1}.day^{-1} kg
BOD_5	687	267	984	116	2924	0.76
COD	905	109	1070	700	3304	0.86

apparent that a strong relationship exists between BOD_5 and COD in the effluent, insufficient simultaneous determinations were made to define a regression equation adequately.

6. Nitrogen and phosphorus forms

Both N and P are important components of freezing works effluent (Table 6-22). Both concentration and loads were high in comparison with the other two effluents. High N concentrations can easily be accounted for by the high protein content of blood and meat residues. The high P concentrations are more difficult to explain. A point worth noting is that only 24% of the TN compared with 52% of TP was in inorganic forms (Figures 6-2 and 6-3). Cleaning compounds would appear to be one possible source of extraneous inorganic P, and in fact have been quoted by Crandall *et al.* (1971) as being the major source of P in U.S. meatworks effluent. An investigation of the cleaning compounds used by Longburn Freezing Company (Peterson Chemicals Ltd., pers. comm.) revealed that of the 3 cleaning compounds used, two did not contain any phosphates while the third contained 9% phosphates. This latter chemical is only used for cleaning operations at the end of the day and is probably an insignificant source as no increases in P concentrations were observed at this time (see Chapter 7).

6.4.4 Colour and ultra-violet absorbance

1. Identification of absorbance peaks

Scans of < 0.45 µm filtered effluent samples (using 1-cm silica cells) revealed that all three effluents absorbed strongly in the far UV (Figure 6-4). Attempts were made to identify the UV peak using a dilution series; these were largely inconclusive. For different samples peaks varied between 210 and 240 nm. In the visible region, sewage effluent samples showed no dominant absorption spectra, dairy shed effluent had a small peak between 660-680 nm, and freezing works effluent displayed somewhat larger peaks between 410-420 nm. Use of 4-cm cells to obtain better definition of the peaks (Figure 6-5) showed that dairy shed effluent displayed a very consistent absorption peak at 671 nm. The absorption peak for freezing works effluent recognised in Figure 6-4 was more variable. The peak varied between 408-418 nm and variation was more apparent between runs than within runs. The reason for this shift may be due to pH differences caused by fellmongery effluent being included or excluded, as pH is known to affect

Table 6-22 Concentration and load of N and P forms in freezing works effluent.

Parameter	Mean concentration ($mg.\ell^{-1}$)	Standard deviation	Maximum	Minimum	Mean discharge.day^{-1} g	Discharge.$hog^{-1}.day^{-1}$ g
TP	6.18	3.15	18.48	1.54	27,810	8.4
TDP	3.23	1.47	8.32	0.73	14,535	4.4
DIP	3.00	1.29	6.86	0.95	13,500	4.1
PTP	2.71	2.32	13.79	0.03	12.195	3.7
DOP	0.25	0.23	1.46	0.01	1,125	0.3
TN	59.0	29.9	119.6	19.0	265,500	80
TKN	58.4	27.7	119.4	16.0	262,800	79
DKN	43.5	22.0	100.0	6.1	195,750	59
NH_4-N	18.6	9.1	45.0	5.5	83,700	25
NO_3-N	0.3	0.2	0.8	0.0	1,350	0.4
TDN	43.8	24.3	89.3	6.2	197,100	59
TPN	14.8	6.7	40.3	11.5	66,600	20

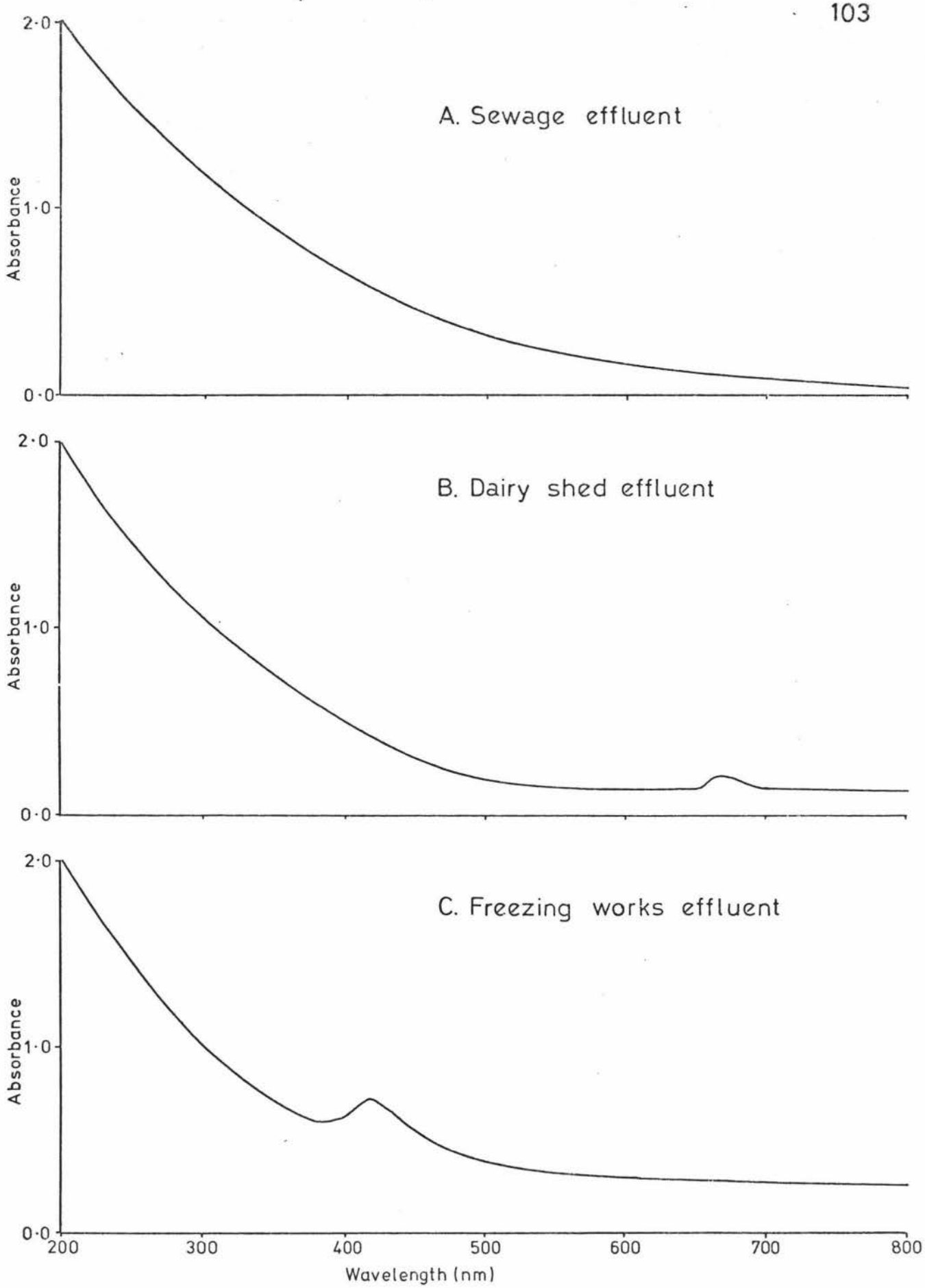


Figure 6-4 Typical scans of effluent samples using 1 cm silica cells

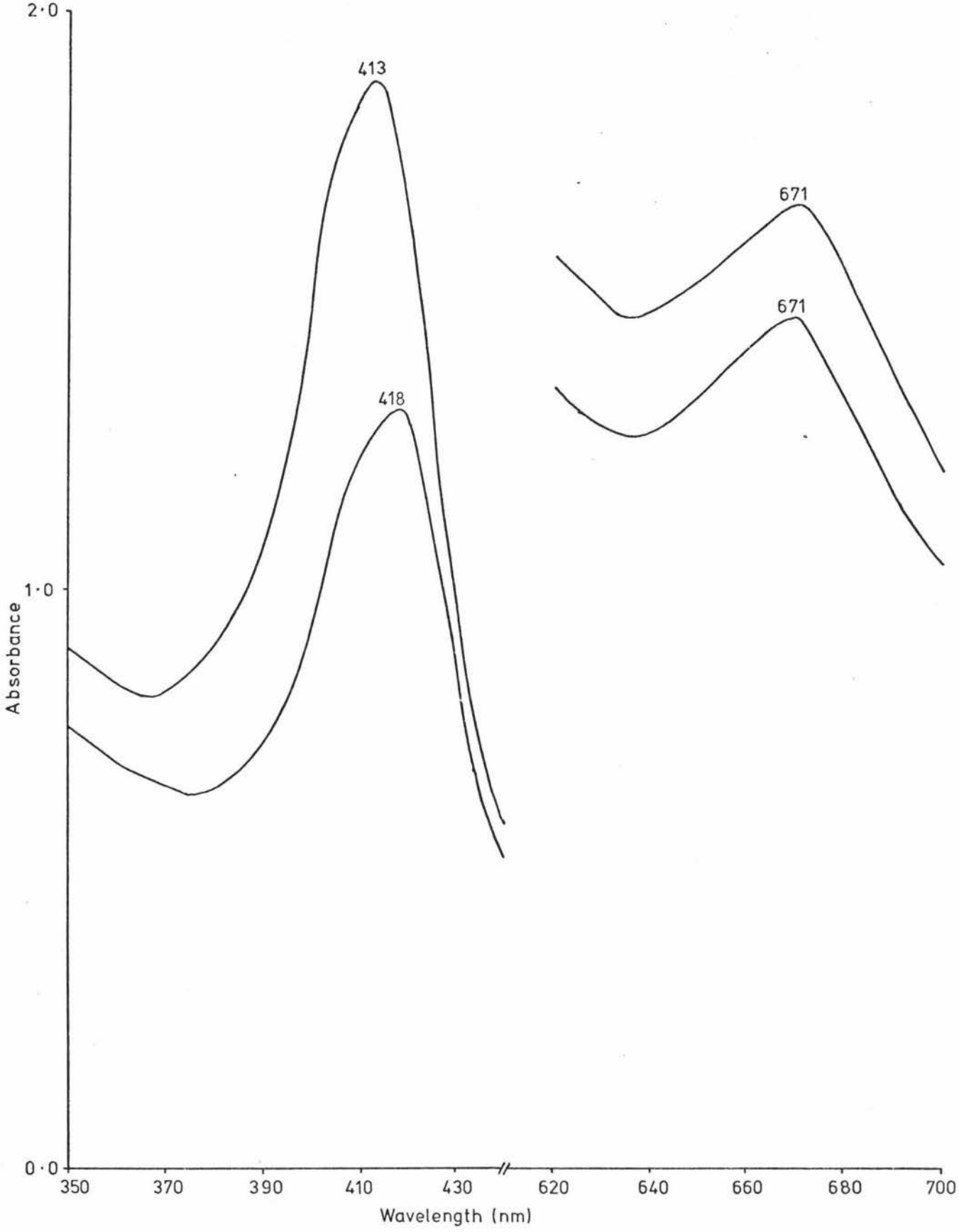


Figure 6-5 Identification of visible absorbance peaks in freezing works and dairy shed effluents using 4 cm cells

colour in waters and wastes (APHA, 1971). No 'visible' absorption peaks could be isolated for sewage effluent except for 4 samples collected between 0000 and 0300 on 10th August, 1976. These samples had a pinkish hue which gave rise to an absorption peak at 490 nm, and may have been caused by discharge of dye wastes from a local knitting mill.

For general monitoring purposes, UV absorbance was measured in 1-cm silica cells at 250 nm (A_{250nm}) for all effluents. 'Colour' absorbance was measured at 413 nm (A_{413nm}) in 1-cm cells for freezing works effluent and 671 nm (A_{671nm}) in 4-cm cells for dairy shed effluent.

2. Correlations between 'colour' and UV absorbance and other analytical parameters

I. Sewage effluent

As no visible absorption peaks occurred with sewage effluent only UV absorbance was routinely monitored. Absorbance at 413 nm was measured for the first sewage sampling run to compare with freezing works effluent. The results indicated that readings at this wavelength were proportional to those at 250 nm, as a correlation coefficient of 0.99 was obtained.

Correlation coefficients between A_{250nm} and each of the other analytical parameters studied (Table 6-23) revealed a number of very strong correlations. The strongest and most consistent correlations occurred with the organic parameters and oxygen-demanding organics (Figure 6-6). High correlations with inorganic parameters (e.g. DIP) are probably coincidental. No positive explanation can be given to the poor correlations obtained in the second sampling run. Stormwater overflow into the sewers could not have caused the anomally as rainfall during this sampling period was light (Appendix 4). A possible explanation is that measurement of A_{250nm} may have been affected by turbidity, due to growth of bacteria in samples left at room temperature overnight.

II. Dairy shed effluent

Correlation coefficients (Table 6-24) indicated that both UV absorbance (A_{250nm}) and visible absorbance spectra (A_{671nm}) could be used to predict a variety of organic or organically-related parameters. In nearly all cases A_{250nm} was superior to A_{671nm} as indicated by the

Table 6-23 Correlation coefficients between A_{250nm} and various analytical parameters in sewage effluent.

Parameter	Sampling run number		
	1	2	3
Flow	0.57	0.16	0.45
TP	0.40	0.07	0.75
TDP	-0.07	0.01	0.78
DIP		-0.02	0.83
DOP		0.09	0.60
PTP	0.71	0.15	0.08
TKN	0.70	0.07	0.88
DKN	0.82	0.17	0.84
NO_3-N		0.10	0.02
NH_4-N		0.04	0.87
TDN		0.18	0.84
TPN		-0.26	0.55
TN		0.08	0.87
BOD_5	0.85		
COD	0.90		0.96
DO			0.05
TC	-0.51		
FC	-0.37		
FS	0.32		
TS		0.41	0.82
pH		0.08	-0.03

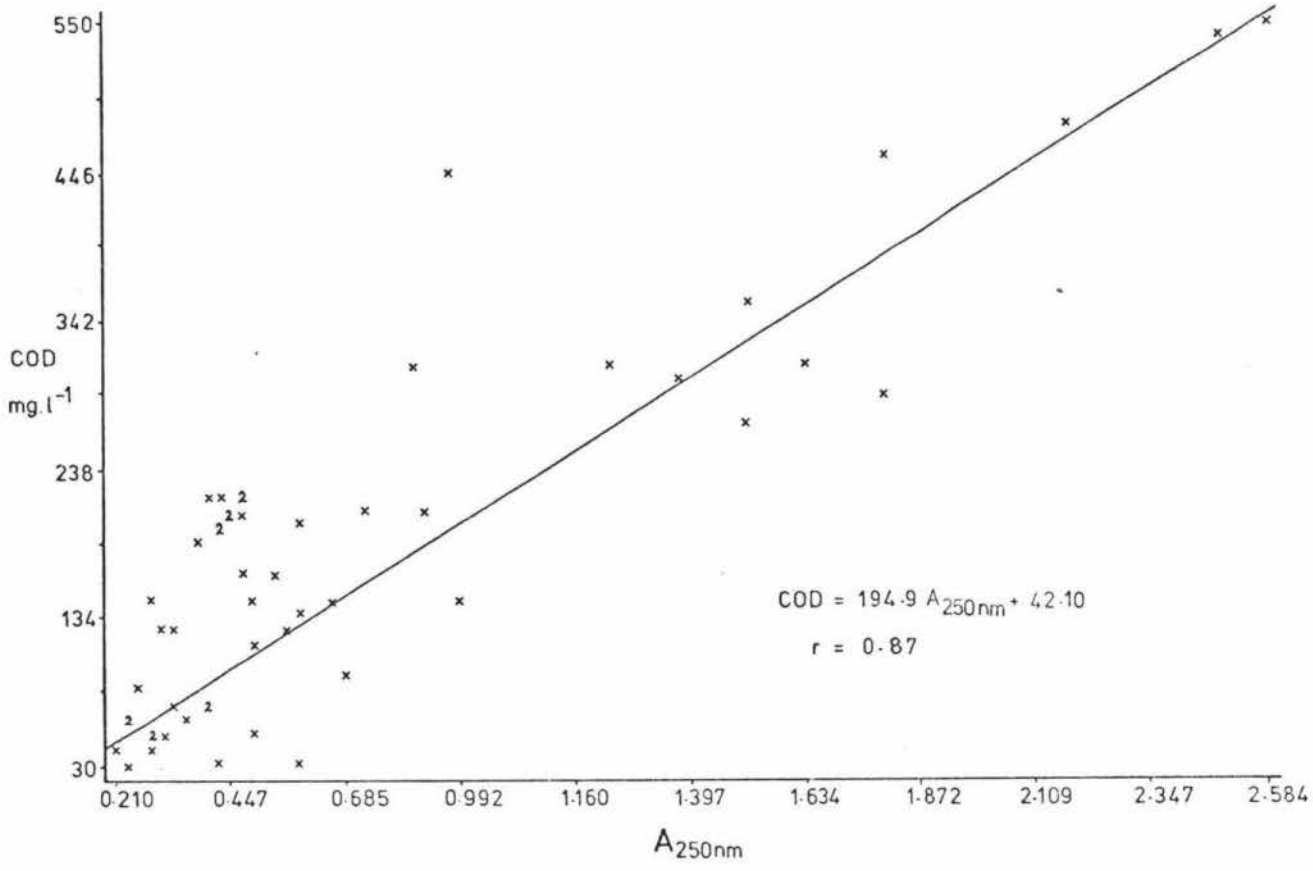


Figure 6-6 Relationship between A_{250nm} and COD in sewage effluent samples (combined sampling runs).

Table 6-24 Correlation coefficients for A_{250nm} and A_{671nm} with various analytical parameters in dairy shed effluent.

Parameter	Sampling run number					
	1		2		3	
	A_{250nm}	A_{671nm}	A_{250nm}	A_{671nm}	A_{250nm}	A_{671nm}
Flow	0.15	0.35	0.39	0.28	0.35	0.05
TP	0.77	0.65			0.75	0.71
TDP	0.62	0.71			0.71	0.71
DIP	0.77	0.70			0.78	0.77
DOP	0.40	0.52			0.47	0.47
PTP	0.57	0.38			0.61	0.58
TKN	0.93	0.85			0.95	0.84
DKN	0.89	0.85			0.93	0.67
NO_3-N	-0.56	-0.48			0.77	0.51
NH_4-N	0.89	0.64			0.84	0.65
TDN	0.88	0.85			0.93	0.67
TPN	0.90	0.73			0.89	0.91
TN	0.94	0.85			0.95	0.83
BOD_5	0.85	0.77	0.90	0.65		
COD	0.87	0.70	0.80	0.69		
DO			0.38	0.24		
TC	0.84	0.62			0.68	0.65
FC	0.93	0.69			0.93	0.87
FS	0.81	0.49			0.84	0.69
TS	0.83	0.60			0.72	0.71
pH	0.27	0.18			0.44	0.51

Table 6-25 Correlation coefficients for A_{250nm} and A_{431nm} with various analytical parameters in freezing works effluent.

Parameter	Sampling run number					
	2		3		4	
	A_{250nm}	A_{413nm}	A_{250nm}	A_{413nm}	A_{250nm}	A_{413nm}
Flow	0.62	0.60	0.75	0.70		
TP	0.76	0.69	0.88	0.76		
TDP	0.54	0.58	0.47	0.40		
DIP	0.56	0.61				
DOP	-0.10	-0.10				
DIP	0.71	0.60	0.92	0.80		
TKN	0.83	0.82	0.98	0.93		
DKN	0.87	0.84	0.98	0.94		
NO_3-N	0.37	0.39				
NH_4-N	0.64	0.66				
TDN	0.87	0.84				
TPN	0.39	0.45	0.63	0.56		
TN	0.82	0.82				
BOD_5					0.72	0.55
COD					0.65	0.14
DO					-0.67	0.05
TC			-0.48	-0.44		
FC			0.67	0.47		
FS			0.38	0.25		
TS	0.90	0.80	0.90	0.81		
SS	0.15	0.13				
pH	-0.07	-0.09				

higher correlation coefficients. A feature of the results is the consistency of the correlation coefficients between sampling runs (Table 6-24). The very high correlations between A_{250nm} and organic or predominantly organic parameters is a very useful result from a practical standpoint. The correlation graphs (Figure 6-7) for combined sampling runs (all dairy shed data combined into one data pool) indicated that A_{250nm} may be used as a predictor for concentration of a variety of parameters used in water quality surveillance programmes.

III. Freezing works effluent

The correlation coefficients for sampling runs 2, 3, and 4 (Table 6-25) show that while UV absorbance gave better correlation with dominantly organic parameters (PTP, TKN, DKN, TS, BOD_5, COD, TC, FC, and FS), absorbance at 413 nm gave a better correlation with dissolved inorganic constituents (DIP, NH_4-N, NO_3-N). These correlations were too weak (0.6-0.7) for accurate prediction. UV absorbance at 250 nm appears to have value as a predictor of Kjeldahl-nitrogen (TKN and DKN) and TS in particular (Figure 6-8) and possibly TP.

IV. Combined effluents

Correlation coefficients were calculated using data files from all effluent sources for parameters which generally showed high correlation coefficients with A_{250nm}. Computed correlation coefficients (Table 6-26) show that although correlations were still quite strong for TP, TKN, DKN, COD, and TS, far better correlations were obtainable on a single effluent basis.

Table 6-26 Correlation coefficients for A_{250nm} with selected analytical parameters in combined effluent files.

Parameter	Correlation coefficient	Parameter	Correlation coefficient
TP	0.64	COD	0.73
TDP	0.46	TS	0.68
TKN	0.74	TC	-0.32
DKN	0.70	FC	-0.22
BOD_5	0.52	FS	-0.22

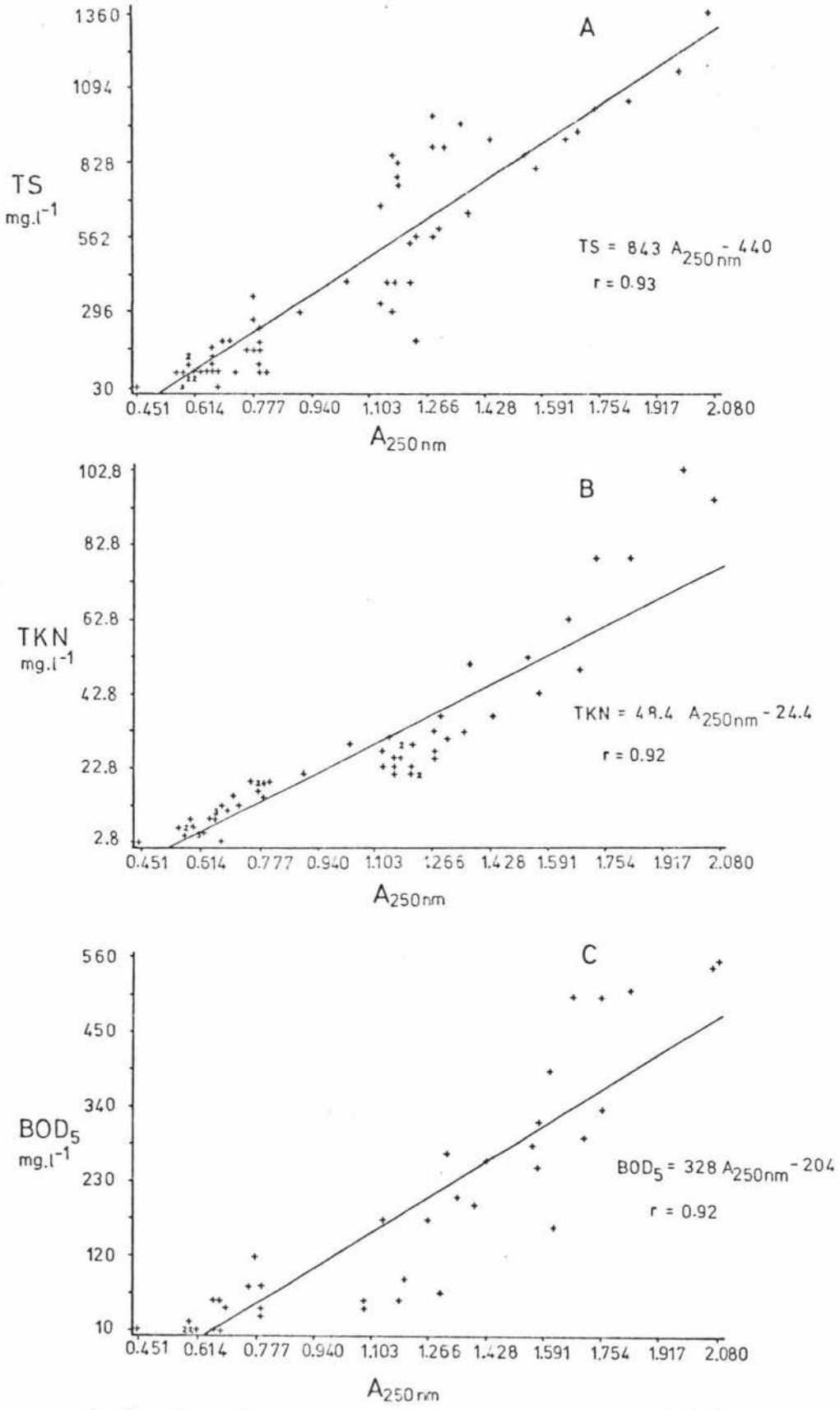
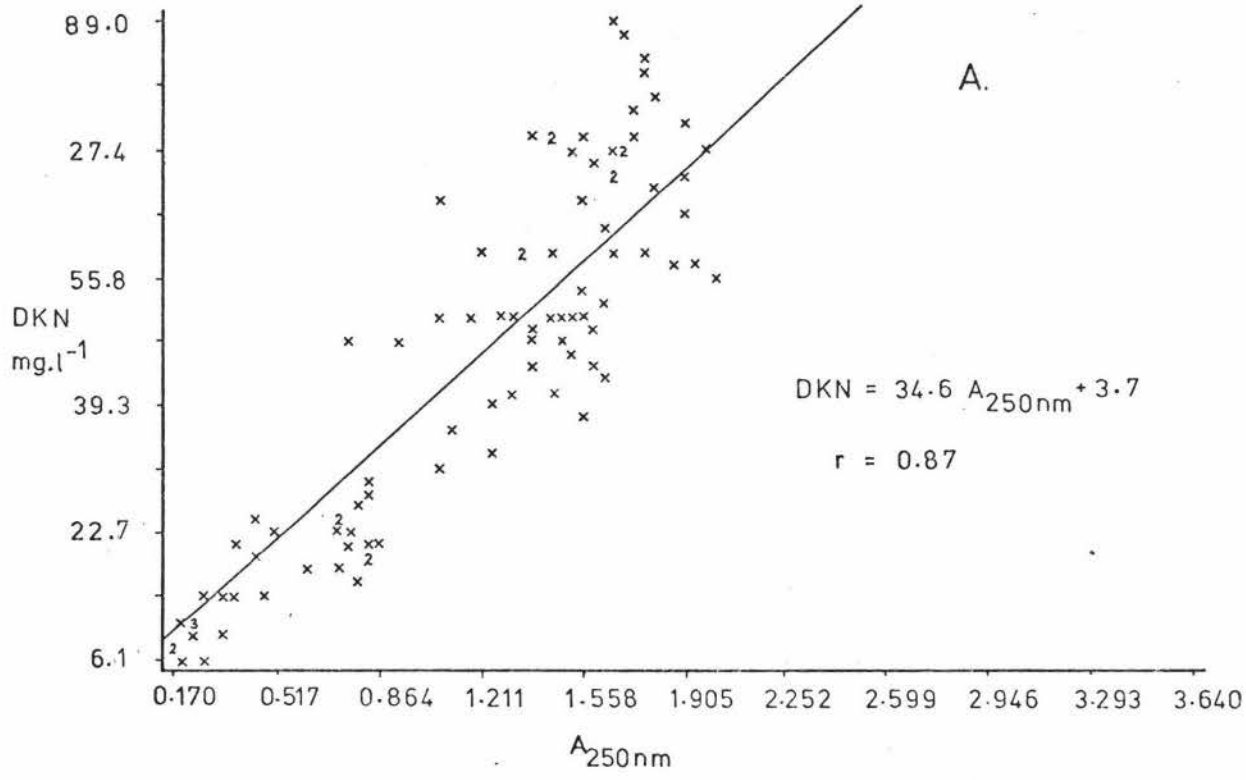


Figure 6-7 Relationship between A_{250nm} and TS(A), TKN(B), and BOD_5 (C) in combined dairy shed effluent sampling runs.



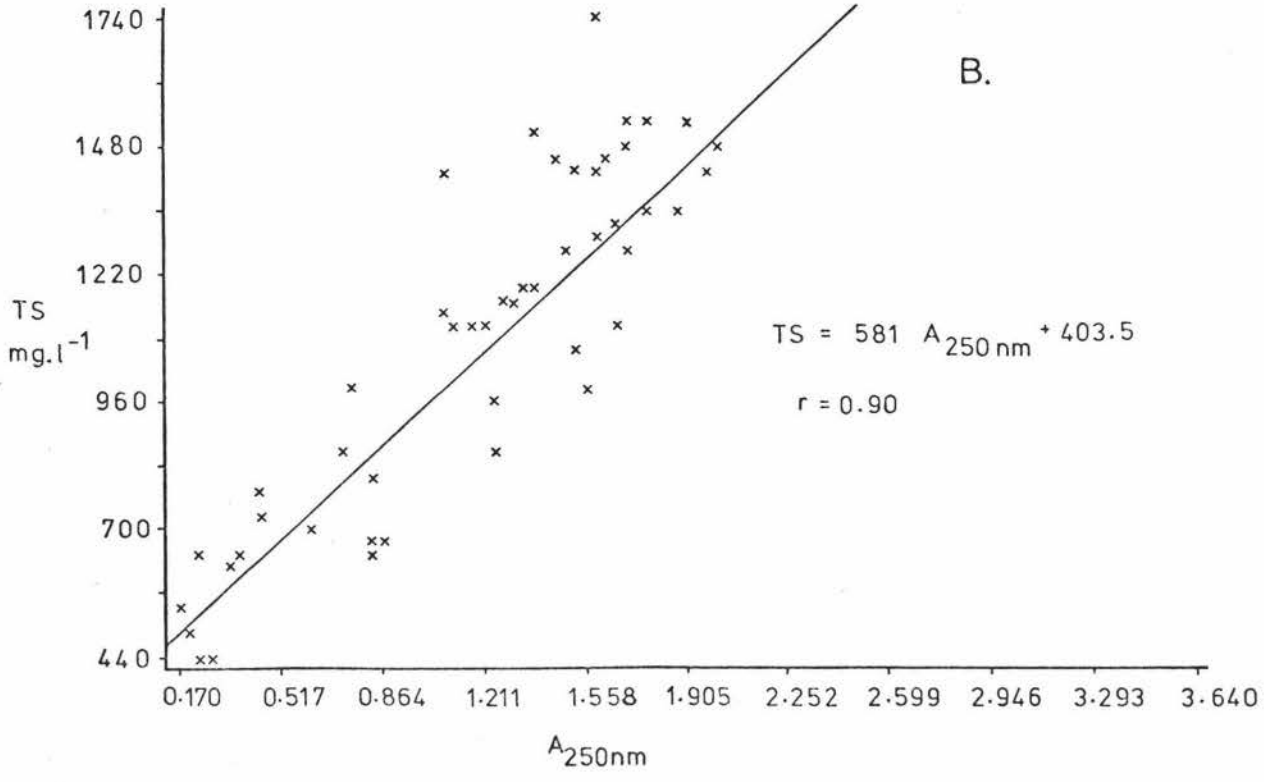


Figure 6-8 Relationship between A_{250nm} and DKN (A) and TS (B) in freezing works effluent samples.

6.5 General Discussion

6.5.1 Effluent composition

Although comparisons between different effluent types which involve different methods of treatment is difficult, some observations of the relative 'pollutional capability' of the effluents on a receiving water are possible. Of the three effluents in this study, freezing works must potentially be considered the most 'polluting'. This assessment is made on the basis that this effluent;

(i) was the only one to have significant deviations from neutrality with respect to pH,

(ii) had the highest mean concentration of solids, bacterial, oxygen-demand, and nutrient parameters,

(iii) had the highest mean daily load of solids and BOD_5, and

(iv) had the highest loading per unit of activity producing the effluent (head of population^{-1}, cow milked^{-1}, hog equivalent slaughtered^{-1}, for sewage, dairy shed and freezing works effluents, respectively) for all of the parameters measured (except DO and NO_3-N).

Municipal sewage effluent contributed 3-4 times more effluent to the Manawatu River in 24 hours than did the freezing works, which in turn was approximately 100 times the volume of the dairy shed effluent. Thus the pollutional capability of the sewage effluent is due to its high total daily loading of solids, nutrients, and oxygen-demanding organics. Although no supporting data (except for high FC densities) are available in this study, it is probable that sewage effluent contributes the most human pathogenic organisms to the river.

By contrast with the other two effluents, the dairy shed effluent is almost insignificant in terms of potential pollutional effect on the Manawatu River. It should be remembered, however, that while there is only one input each of sewage and freezing works effluents into the Manawatu River, there are many contributions from dairy sheds. In addition, most of these effluents discharge into small farm streams or ditches before eventual discharge into the Manawatu, possibly causing similar damage to these waterways as are the major discharges into the Manawatu. A feature of the dairy shed effluent was the sudden discharge of a 'slug' of very concentrated effluent over a short time period (see Chapter 7). Despite the occasionally concentrated nature

of the discharge it is evident that the total amount of oxygen-demanding organics and nutrient forms discharged in 24 hours by the milking of one cow (Table 6-16 and 6-17) is considerably less than that discharged by the activities of one urban human in the same period (section 6.4.1).

6.5.2 Evaluation of oxygen-demand parameters and faecal indicator bacteria for the characterisation of effluent discharges

1. Oxygen demand

The question of which test to use as a parameter for the routine monitoring of oxygen demand has been discussed by a number of workers (Gaudy, 1972; Humerick *et al.*, 1974). All too often it appears that the BOD_5 test is selected for a monitoring programme, purportedly because of its biological implications, but without any real thought of a biological interpretation of the resulting data.

Where an oxygen-demand analysis on an effluent is to be used for the prediction of DO depletion in the receiving water, then the use of a biological rather than a chemical test has obvious advantages, as the only substrate to be utilised is that causing the oxygen depletion. The limited success achieved overseas in applying the Streeter-Phelps principle to model DO in receiving waters (Ackerman *et al.*, 1974), however, underlines the fact that it is a field beyond the technical capabilities of most Regional Water Boards. In fact the BOD_5 test is generally used by RWBs not as a means for predicting DO but rather as an absolute measure of the 'strength' or 'polluting potential' of the effluent. BOD is in effect used to measure the substrate concentration of the effluent, and although this is closely related to DO prediction the two uses are not synonymous.

The chemical-oxygen demand test (COD) provides a superior method of measurement of substrate concentration as it:

(i) achieves a higher degree of oxidation than does the BOD_5 test and

(ii) is unaffected by the varying oxidation rates of effluents, unlike BOD_5 which may require the addition of seed and nutrients.

Although the BOD_5 and COD are completely different tests (APHA, 1971), for any particular effluent source there exists a very strong relationship between BOD_5 and COD (Figure 6-1). Therefore it is possible to predict the readily biologically oxidizable component of

the effluents from COD data with a reasonable degree of accuracy. Similar relationships to those in Figure 6-1 have been derived by Viraraghavan (1976).

Other technical advantages of the use of COD rather than BOD_5 for RWBs are:

(i) the COD test is simpler, quicker (3 hours for the manual test), and more reproducible (Humenick *et al.*, 1974),

(ii) preservative techniques can be used for samples prior to COD determinations (Benedek and Najak, 1975), thus facilitating transportation to a central laboratory where automated analysis is available, and

(iii) where substrate concentration is used as a basis of effluent charges (a future possibility), the use of COD provides financial incentive for industry to use materials that are degradable, whereas BOD encourages the use of inhibitory or toxic compounds (Dart, 1977).

It may be argued that the expense of the COD test (use of silver sulphate as a catalyst) may lessen its attraction to enforcement agencies. Recent studies (Clark and Mitchell, 1975), however, indicate that the concentration of silver sulphate may be reduced by two-thirds without significantly decreasing oxidation.

2. FC:FS ratio for differentiation between human and non-human faecal pollution sources.

The FC:FS ratios for freezing works and dairy shed effluents obtained in both the monitoring study and the growth/die-off experiments (Chapter 5), are in conflict with the scheme proposed by Geldreich and Kenner (1969). According to this scheme both effluents would be catagorised as being derived from human sources. There are three possible reasons for this anomally:

(i) Differential die-off growth between faecal coliform and faecal streptococci sub-groups,

(ii) The scheme proposed by Geldreich and Kenner (1969) is not applicable to these effluents, and

(iii) A systematic experimental error in the present study.

The first possibility has some merit as it has been reported (McFeters *et al.*, 1974) that enterococci (dominant FS group in human faeces) survive better than FC, which survive better than *S. bovis* and

S. equinus (dominant FS group in cattle and pig faeces). The latter group is known to have a very rapid die-off rate outside the intestinal tract of the animal (McFeters *et al.*, 1974). Feachem (1975) claimed that because of the different survival characteristics of these bacterial groups, the FC:FS ratio should tend either to fall or rise with time in human and non-human faecal wastes, respectively. The results of the growth/die-off experiments (Chapter 5) are not consistent with the above arguments. The FC:FS ratio in dairy shed effluent (which theoretically should have *S. bovis* and *S. equinus* as the dominant FS sub-group) decreased rapidly with time (Figure 5-4) due to the rapid increase in FS numbers. Similarly the FC;FS ratio in sewage effluent rose with time while the ratio in freezing works effluent stayed approximately constant.

Recent work on the same dairy shed effluent that was used in this study (Guy and Small, 1977) reinforces both aspects of the argument given above. Guy and Small (1977) reported that the levels of faecal streptococci in samples of drainage waters (from the effluent disposal site) were lower than those of faecal coliforms (FC:FS > 1). In contrast to the experiments in this study (Chapter 5), Guy and Small (1977) observed preferential increases in levels of faecal coliforms over faecal streptococci in their longer term (5-7 day) survival experiments. Small increases in FS numbers in the short term (24 hr), however, were observed by the above authors. Further confusion is added in this same study by the report that *S. bovis* (which theoretically has the fastest die-off rate of all the indicator bacteria) comprised 80% of all isolates from the soil-filtered effluent samples 4 hours after collection, and that after 4 days of storage this species still comprised 18-55% of the original at all storage temperatures.

The report by the above authors that FC numbers were higher than FS must belie the scheme of Geldreich and Kenner (1969) for this particular effluent. Although different media were used in the present study from that used by Guy and Small (1977) the possibility still exists that the MF method may give rise to a systematic experimental error for the determination of the FC:FS ratio in animal faecal wastes. This experimental error may have been induced by either overestimation of faecal coliforms or underestimation of faecal streptococci. Pyle (1974) has suggested that high organic matter may lead to overestimation

of faecal coliforms by the MF method, though no explanation of possible reasons was advanced. In the present study, the underestimation of FS is more likely. Geldreich (1976) tabulated results from various sources which showed that the use of M-enterococcus agar (used in this study) seriously underestimated FS populations when compared with KF agar. The findings indicated that KF agar was more sensitive for detecting faecal streptococci in general and *S. bovis* and *S. equinus* in particular. The use of;

(i) KF agar for the determination of FS,
(ii) the MPN method to calibrate the MF method for faecal coliforms (Coliform Committee, New Zealand Microbiological Society, 1976), and
(iii) confirmatory tests,

would assist in determining the existence of any systematic experimental error.

Although the disparity between the results of this study, together with those of Guy and Small (1977), and the scheme proposed by Geldreich and Kenner (1969), may have been caused by any of the reasons discussed above, it is evident that;

(i) caution must be exercised in interpreting the FC:FS ratios from effluent sources, and
(ii) further work and recommendations as to the methods and media required for determination of both FC and FS in effluents are justified.

6.5.3 Use of visible and UV absorbances as an index of pollutional parameters in effluents

The results of this study indicate that A_{250nm} may be used to develop accurate prediction equations for a variety of pollutional parameters in effluents. Those parameters which showed a generally strong correlation with A_{250nm} for all effluents are: TP, TKN, DKN, NH_4-N, TDN, TN, BOD_5, COD, and TS. Most of the other parameters had a strong correlation in one or more effluents. DOP, NO_3-N, DO, and pH displayed only weak or no correlation with A_{250nm} in all effluents.

The results of this study are difficult to compare with others because to the knowledge of the author no other study has examined the correlation between A_{250nm} or visible absorbances with such a wide range of analytical parameters and effluent types. UV absorbances

within the range 210-300 nm have been used as an index of: dissolved organic matter (DOM) in seawater (Foster and Morris, 1974; Balch *et al.*, 1975), surface waters and wastewater (Mrkva, 1969, 1975); total organic carbon (TOC) in surface and wastewaters (Dobb *et al.*, 1972); and also organic groups characteristic of specific industrial discharges (Mrkva, 1969; Timperley, 1975). Strong correlations between A_{250nm} and NO_2-N, P, Log TC, and Log FC ($r = 0.81-0.87$) in seawater near sewage outfalls were reported by Balch *et al.* (1975). In common with the present results, only weak correlations were found with NO_3-N which is known to absorb in the UV at 203 nm and 302 nm (Dobbs *et al.*, 1972). The strongest correlations in the present study were obtained with parameters reflecting a solely or largely organic character. Also correlations were strongest in dairy shed effluent and weakest in sewage effluent. This may reflect a decreasing degree of unsaturation and aromaticity in the organic components from dairy shed effluent to sewage.

If further research bears out the observed correlations then A_{250nm} could be a very valuable tool for enforcement or compliance monitoring. Individual correlation equations between A_{250nm} and selected parameters could be developed for each type of effluent. Checks with additional effluents of the same type could be used to verify the relationship, and if supported, absorbance at 250 nm could be used as a monitoring aid for enforcement agencies. The initial financial outlay for a spectrophotometer with UV capabilities would soon be negated by the large saving in time and money required for analysis of present pollutional indicators.

The use of absorbance at specific visible wavelengths, i.e., A_{413nm} for freezing works effluent and A_{671nm} for dairy shed effluent has met with limited success. In general A_{250nm} proved superior in terms of correlation coefficients for the parameters studied, although correlations were still very high for a number of parameters with A_{413nm} and A_{671nm}. The higher correlation of A_{413nm} with inorganic N and P parameters in freezing works effluent compared with A_{250nm} deserves further investigation.

CHAPTER 7 EVALUATION OF SAMPLING FREQUENCY REQUIRED TO CHARACTERISE EFFLUENTS

7.1 Introduction

The value of an analysis (chemical, physical, or biological) on an effluent (or receiving water) is no greater than that of the sampling programme employed. If a sample is taken without due regard to the flow conditions, the variability of the parameters under consideration, and the site from which the sample is taken; then the results of any subsequent analyses lose most, if not all, of their significance.

The results of the survey (Chapter 2) indicated that few Regional Water Boards implemented sampling 'programmes' for effluent discharges, and that most sampling was done on a haphazard basis. While this is undoubtedly due to a lack of resources in most cases, it is clear that few guidelines are available to assist Regional Water Boards in planning a sampling programme for effluents. In addition, little data are available in New Zealand on the variability of chemical and microbiological parameters in municipal sewage or effluents from agriculturally-based industries.

In this chapter the data on time- and flow-based variations in the composition of sewage, freezing works, and dairy shed effluents are analysed by two different methods in order to determine the sampling frequency needed to characterise effluent discharges.

7.2 Review of Literature

The most important requirement for a satisfactory sample is that it is representative (Mancy and Weber, 1971). There are many subtleties, both mathematical and physical, in this word 'representative'. The composition of the sample should be identical to that of the effluent from which it was collected (King, 1971). In other words the method of sample collection should not impair the physical or chemical composition of the sample. Several types of automatic samplers (Little, 1973) do just this. By having slow pumping rates suspended solids are inclined to settle out in the collection tube, and subsequent determinations of suspended solids are underestimated. For this reason, Jenkins (1976) advocates isokinetic sampling. That is, the body of fluid is captured at its natural velocity.

The problems of obtaining a physically representative sample are compounded when untreated wastewater is to be sampled. For example, no 2-litre sample of raw sewage is likely to be truly representative of the bulk liquid. If it contains no large characteristic sewage solids, then it is clearly unrepresentative of the bulk material which does contain solids; if it contains one such solid then it is likely to contain more than the average two litres of liquid. Short of breaking down the solids in a fairly large volume and sampling that, it is impossible to remedy the situation. The Department of the Environment (G.B.) (1972) recommended that sampling at a sewage works should not be done before the screening stage.

Even treated effluents contain a proportion of readily settleable solids, however, and sampling should be conducted at a point of considerable turbulence so that the correct proportion of suspended matter can be achieved (Mancy and Weber, 1971; Montgomery and Hart, 1974).

The physical size of a representative sample is dependent on the concentration of the measurement parameter in the sample (Allen, 1971; King, 1971). For example, determination of phytoplankton biomass in an oligotrophic lake will require much larger sample volumes than will the same measurement in a sewage lagoon.

In the mathematical sense, for a sample to be representative it must be a valid representation in terms of time and space. For

this to be so the sample should strictly be selected by a process of random selection (Allen, 1971; Mancy and Weber, 1971). According to these authors, random selection is one of the most basic, yet frequently violated principles in the development of a sampling programme. They further claim that by neglecting the principle of random selection, any statistical evaluation of the data is impaired.

While agreeing that random sampling is statistically the most correct method for sampling streams of effluents, Rainwater and Avrett (1962) postulated that because stream composition is continuous and non random, sample statistics computed from a given number of systematic measurements will more closely describe the stream population than an equal number of random measurements. These authors proposed a procedure in which the 'probable efficiency coefficient', computed from the differences in sequential algebraic signs of the sample measurements above and below the sample median, was used to reflect the equivalent number of measurements from a random series. By use of this coefficient, statistical procedures which are strictly applicable only to random samples can be used. Montgomery and Hart (1974) have used a similar procedure to show that systematic sampling is more useful for characterising effluents than is random sampling. The benefits of systematic sampling decrease as the parameters being measured increase in variability (random fluctuations). Care must be exercised to ensure that the systematic sampling is not in phase with a cyclic fluctuation. For example, McCoy (1971) showed that variations in concentration of faecal indicator bacteria in sewage are the result of the pattern of defaecation in the community. Between 50 and 60% of the daily bacterial load in raw sewage occurred between 6.00 a.m. and noon, and between 15 and 20% in the period noon to 6.00 p.m. Thus if only two samples were taken at 12 hourly intervals during a 24 hour period, the computation of bacterial loading would be seriously under- or over-estimated, depending on the time of the initial sample. Where summary values are needed (mean, median, etc.), 6 samples.cycle^{-1} has been suggested (Montgomery and Hart, 1974) as a working frequency for defining a cyclic variation.

When samples are taken at equal intervals, at a frequency great enough to allow for all important fluctuations in concentration to be sampled representatively, the data may then be regarded as time

based. Similarly, if samples are taken at equal intervals of flow with the same criteria regarding frequency, the data may be regarded as flow based (Little, 1973). This latter system of sampling is biased in favour of large flows and in situations where high concentrations occur with low flows, the average concentration is much lower than that calculated from the overall average.

The problem of obtaining a valid representative sample of an effluent stream has been overcome in some instances by the use of composite samples. This type of sample may be time dependent, flow dependent, continuous, or continuous at rates proportional to flow. A composite sampler of the latter type was described by Little (1973) who claimed that more representative samples could be obtained with this than with other types of samplers. One of the simplest types of composite samplers, the air displacement sampler, was described by Jenkins (1976). The sampler chamber is immersed in the fluid so that hydrostatic pressure due to the depth of liquid pushes the sample in. The rate at which this is allowed to happen is adjusted by a regulator, which controls the rate at which air can escape. This type of sampler has the advantage of being isokinetic and hence is especially suited for suspended solids sampling. Composite sampling has many advocates. Theroux *et al.* (1943) preferred composite sampling for sewage influent and effluent because grab sampling was unreliable. Grab samples, however, are generally recommended for parameters which require immediate analysis to be meaningful (Env. Sci. & Tech., 1974). Bulking of samples is not valid for analyses such as dissolved oxygen, temperature, pH, or dissolved and particulate constituents (as opposed to total), whose concentrations may be affected by the bulking process. Biological parameters, such as indicator bacteria and BOD_5 which change rapidly with time, are also of dubious significance when determined on a composite sample. Refrigeration of the composite sample will help to minimise changes. Composite sampling is most useful for determining average conditions (Little, 1973; Montgomery and Hart, 1974) and especially for determining stable parameters (Crowley, 1972). A statistical comparison of results obtained from composite and grab (3 systematic samples per day with time of initial sampling varied) sampling on two different effluents (Tarazi *et al.*, 1972) showed that grab sampling did not give significantly different

results for an effluent in which flow was relatively constant. There were significant differences, however, for an effluent with wide variations in flow. In this respect their statistical comparison of loads was undoubtedly affected by only using the discrete flow values obtained at the time of sampling. As they reportedly used computer techniques for making the comparisons, use of the continuous flow record or integrating a larger number of discret flow values would have given a better estimate of load. Tarazi *et al.* (1972) strongly favoured composite sampling on the basis of their comparison.

Apart from analytical considerations, grab sampling has one important advantage over composite sampling and that is its ability to provide some measure of the variation of constituents in the effluent (Little, 1973). The value of grab sampling (better called 'discrete') is reflected in its popularity (Rainwater and Avrett, 1962).

Because it is necessary for pollution control purposes to rely largely on the data obtained by intermittent discrete sampling and subsequent analysis, a compromise must be made in which the accuracy of the information gathered is balanced against the frequency of sampling, and the attendant labour and costs. A determination of the desirable frequency of sampling is dependent upon the type of information required (Little, 1973; Briggs, 1976). The purpose of any sampling programme may be divided into:

(i) a representation of the whole bulk of uniform material,
(ii) an indication of fluctuations in concentrations with an estimate of the average on a variable load,
(iii) a measure of the pollutional load, and
(iv) the location of high or low conditions (in concentration and/or load) in a large volume of material.

The number of samples needed to observe trends will be less than the number needed to define variations and locate maximum and minimum values. In the latter case, the frequency of sample collection is directly related to the variability of the constituents being analysed and to the level of significance specified (King, 1971).

If the concentration of the parameter under consideration in an

effluent is uniform and flow is steady, then a single sample taken at any time will be representative. Usually the effluent is nothing like uniform in composition and flow is variable. In this case the relationship between the composition of a single sample and the average composition is a matter of probability.

The use of existing data to estimate statistically the frequency of sampling is the most frequently used method. In many cases, discrete values of concentration or load, as measured by sampling and analysis, are distributed according to statistical patterns with well-established properties, such as the normal and log-normal probability curves (Montgomery and Hart, 1974). Advantage can be taken of these patterns to estimate the sample standard deviation ($\hat{\sigma}$) of the population standard deviation (σ). From this statistic, and by choice of the confidence level (C), and the precision (p) with which the result must be known, the number of samples ($\underline{N}$) required to establish a given mathematical result may be estimated (Montgomery and Hart, 1974). For example, the number of samples required to establish the arithmetic mean of data having a normal distribution is:

$$\underline{N} = \left[\frac{k\hat{\sigma}}{p} \right]^2$$

where k is a constant, the value of which is dependent upon the confidence level chosen.

It can be seen that p is related inversely to $\sqrt{\underline{N}}$, hence in order to keep $\underline{N}$ to a minimum, p should be made as large as permitted by the objectives of the sampling programme.

A similar approach involving the statistical distribution of data was used by the United States Environmental Protection Agency (1975) in a method for effluent compliance monitoring. This computerised method, which is designed for use by enforcement agencies, uses the nature of the distribution, along with estimates of the cost of a potential violation and a rationalisation of the resources available to monitor the effluents. The output of the program is a compliance monitoring schedule for the agency to follow.

Where the objective of the sampling programme is to determine the total load of the effluent stream a less rigorous approach, such

as that used by Sharpley *et al.* (1976), may be suitable. In this method the variation in total load caused by deletion of certain sampling intervals as a percentage of the load measured with all sampling intervals present is determined (see section 7.3).

From this brief survey of the literature it is clear that there is a shortage of information on the design of sampling programmes for effluents of different characteristics. Such programmes are required if meaningful data are to be obtained.

7.3 Methods

7.3.1 Computer integration for estimation of loads

The computer program described in Chapter 6 was used to estimate the sampling frequency required to determine the total load of the various analytical parameters considered (Sharpley *et al.*, 1976).

For selected sampling runs, the loads of analytical parameters for which a complete or nearly complete suite of data existed (mainly N and P forms), were calculated using the program. Then cards representing analytical data from different sampling times were progressively subtracted so that the loading program calculated loads based on 1-, 2-, 4-, 8-, 12-, and 24-hour intervals between sampling points. Data cards representing flow were substituted in the card deck so that the total flow calculated remained constant.

Variations in the first 'sampling' data card were made for intervals > 1 hour to see if this affected the variation in total loads.

Because the program is designed to separately average flow and concentration between successive positive data points and calculate load by integration, and because flow was always constant, any variation in the total load of the various analytical parameters must be due to variation in the concentration of the parameters. This indicates the minimum sampling frequency which should be used to estimate load within prescribed limits. A variance of ± 10% of the load calculated with all sampling points present, was chosen as the allowable limit.

7.3.2 Use of statistical distributions for estimation of sampling frequency needed to determine summary statistics

Probability plots of data obtained in sampling runs were constructed (Montgomery and Hart, 1974) and the characteristic in question was assigned to either a normal or a log-normal distribution depending on the fit of the data. Only consistent, systematically sampled data were used. For example, in a sampling run where hourly samples were taken, frequency may have been increased to quarter hourly for some periods. In such cases only the hourly data were used as inclusion of the intervening data may have biased the distribution.

The sample standard deviation ($\hat{\sigma}$) was obtained by computation

using an SPSS system program. $\hat{\sigma}$ may also be estimated (less accurately) by taking half the distance between the 16 and 84 percentile values of x (or log x in a log-normal distribution) read off a straight line fitted to the plotted points by eye (Montgomery and Hart, 1974). The precision (p) was chosen to be 10% (rounded) of the total range in concentration of the constituent in question. Values of constants (f and k) were selected from Tables 7-1 and 7-2, and the number of samples ($\underline{N}$) to establish the arithmetic mean and median was calculated according to the appropriate equation in Table 7-3.

Table 7-1 Values of k for given confidence levels.
(Montgomery and Hart, 1974).

Confidence level (per cent)	99	98	95	90	80	68.3	50
k ...	2.58	2.33	1.96	1.64	1.28	1.00	0.67

Table 7-2 Values of f for given percentiles.
(Montgomery and Hart, 1974).

Percentile	50	40	30	20	16	10	5	1
	(median)	60	70	80	84	90	95	99
f	1.25	1.27	1.32	1.43	1.52	1.71	2.09	3.67

Table 7-3 Number of samples to establish result with precision (p) at required confidence level.
(Montgomery and Hart, 1974).

Result required	Distribution of existing data closer to	
	normal	log-normal
Arithmetic mean	$\left(\frac{k\hat{\sigma}}{p}\right)^2$	Estimate percentile of arithmetic mean from line of best fit, then apply $\left(\frac{kf\hat{\sigma}}{\log.q_2 - \log\bar{x}}\right)^2$
Median	$\left(\frac{1.25k\hat{\sigma}}{p}\right)^2$	$\left(\frac{1.25k\hat{\sigma}}{\log.q_2 - \log D}\right)^2$
A percentile	$\left(\frac{kf\hat{\sigma}}{p}\right)^2$	$\left(\frac{kf\hat{\sigma}}{\log.q_2 - \log D}\right)^2$

where q_1 and q_2 are the lower and upper limits of the existing data.
D is the value of x in the existing data at the percentile under consideration.

7.4 Results and Discussion

7.4.1 Factors affecting the determination of sampling frequency

1. Hydrological

The flow from all three effluent discharges was strongly time dependent. The flow of sewage effluent (Figure 7-1A) was highly predictable unless very intense rain caused parts of the stormwater system to overflow into the sewage system. Some inputs from stormwater occur whenever there is rainfall due to infiltration but these are becoming less significant as both the sewage and stormwater systems are being continually upgraded. Generally, sewage flow decreased between midnight and 6.00 a.m. It then increased rapidly between 6.00 a.m. and 9.00 a.m. and continued to increase, although at a slower rate until approximately 1.00 p.m., at which time peak flow generally occurred. Flow then declined slowly until about 7.00 p.m. at which time flow increased slightly causing a small flat peak in flow between approximately 8.00 p.m. and 11.00 p.m. After midnight flow declined steadily reaching its minimum at 5.00 a.m. to 6.00 a.m. the next morning.

The time of retention in the sewage system was 2-3 hours, being fairly evenly divided between reticulation time and treatment time. Thus any instant on the sewage hydrograph (Figure 7-1A) reflected the activity in the community approximately 3 hours previously.

Discharge of effluent from the Longburn Freezing Company's treatment plant was strongly dependent on working time (Figure 7-1B). A continuous pump kept a small amount of effluent discharging during the night. The flow of effluent increased rapidly when killing operations commenced at about 7.30 a.m. and stayed high, with small fluctuations, until approximately 4.00 p.m. when killing stopped. Post-kill cleaning operations caused the decline in the flow at this time to be far slower than the initial increase. The fluctuations in effluent flow during the day were due to killing activity within the works. The treatment plant is based on an overflow principle so that the amount of effluent leaving the plant is directly related to rate of influent. Thus breaks in killing and subsidiary operations, caused by 'smokos' and lunch breaks were easily detectable on the hydrograph (Figure 7-1B).

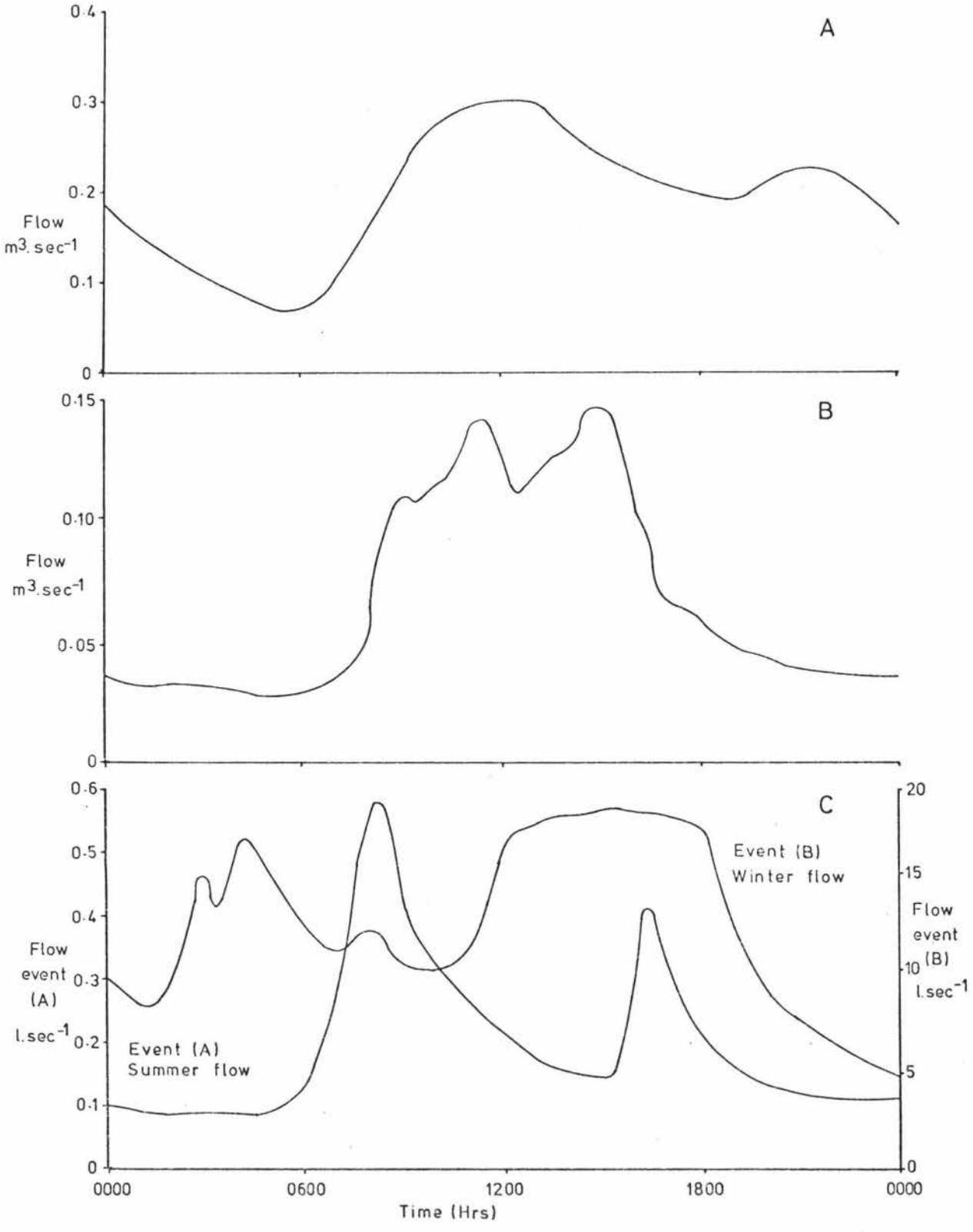


Figure 7-1 Typical hydrographs of the municipal sewage (A), freezing works (B), and dairy shed (C) effluent discharges.

Flow from the dairy shed effluent disposal area (Figure 7-1C) was dependent on milking operations and on soil moisture content, which in turn was dependent on precipitation and evapotranspiration. In summer (or when the soil on the disposal site was below field capacity) the majority of the flow was associated with milking times. The most concentrated period of effluent application was during and just after the shed washdown period. The shape and size of the hydrograph associated with effluent application was dependent on the antecedent soil moisture. The time of concentration at the weir generally varied from 15 minutes to 1 hour, depending on the location of the sprinkler and soil moisture. The volume of effluent measured at the weir in a morning milking event was often greater than for an afternoon event because the sprinkler was moved once in 24 hours, after the morning milking event. Thus the antecedent soil moisture for the morning event was generally higher due to the application of the previous afternoon's effluent.

In late winter and spring when the soil was generally at or close to field capacity (Curve B on Figure 7-1C) the shape of the hydrograph was largely determined by the pattern of precipitation, as is the case with any tile drainage system. If the hydrograph was on the declining limb at the time of effluent application, a small peak due to the effluent may have been recorded at the weir. If the effluent was applied on the rising limb of the hydrograph or during the peak flow period caused by rainfall, however, then usually the effluent application was masked altogether. This was the case for the afternoon milking event on Curve B of Figure 7-1C. During late autumn/mid-winter when the cows were not being milked the hydrograph was entirely dependent on climatic conditions.

2. Time-based variations in concentration and load of analytical parameters

It has already been established that all of the effluent discharges under consideration are time based to some degree. It is therefore apparent that separation of analytical variations into time-based and flow-based variations is very difficult.

The concentrations of chemical parameters in sewage effluent appeared to be proportional to flow (Figure 7-2). pH had a distinct diurnal variation which may or may not be related to flow (Figure 7-2A). pH increased by approximately 0.5 of a unit between

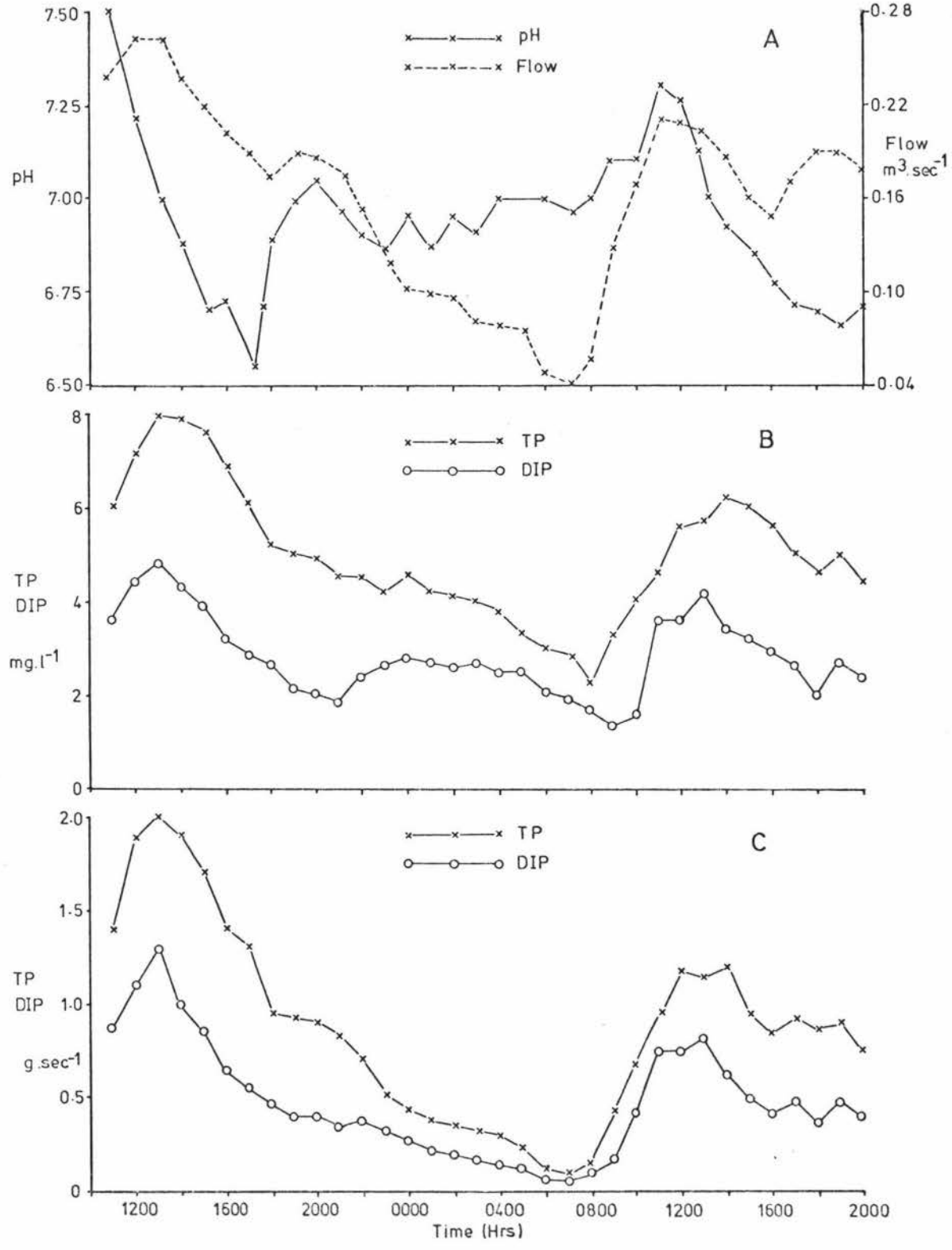


Figure 7-2 Variation in pH (A), DIP and TP concentration (B), and DIP and TP load (C) in sewage effluent sampled on 9-10 August, 1976.

8.00 a.m. and 12 noon each day. This may have been due to the use of detergents for laundry use, etc. TP and DIP concentration (Figure 7-2B) also increased during this time, and these constituents were clearly related to flow. Their respective loading curves (Figure 7-2C) accentuated this correlation. The other N and P forms studied also showed this relationship.

In sampling municipal sewage effluent, the possibility exists that non-representative samples will be taken due to a shock industrial discharge. Such an event was sampled on June 23, 1976 and the initial samples were a dirty brown colour which was due to the discharge of yeast manufacture effluent (sewage treatment plant operators, verbal communication). The high proportion of only slow biodegradable organics in this effluent was reflected in the relative variations in BOD_5 and COD load (Figure 7-3A). In this instance, the concentration of the oxygen-demanding organics appeared to be a more important factor than flow in determining the load of BOD_5 and COD. The relative concentrations (or loads) of BOD_5 and COD converged with time, which was probably a reflection of dilution of the industrial input with a greater proportion of domestic sewage.

This input of industrial effluent may have upset the usual relationship of faecal indicator bacteria with flow. McCoy (1971) commented that the concentrations of faecal indicator bacteria in sewage effluent varied according to a well-defined pattern, regulated by the sanitary characteristics of the community. As the flow and concentration of chemical parameters appeared to follow such a pattern it would seem logical that faecal indicator bacteria should do likewise. Although a weak trend existed (Figure 7-3B & C), however, it was not as clear as for chemical parameters. The presence of yeast manufacture effluent in the early stages of sampling may have adversely affected the survival of faecal indicator bacteria.

The concentrations of chemical parameters in freezing works effluent were proportional to flow (Figure 7-4), although peaks in concentrations were not always in phase with peaks of flow. The particulate fractions of P and N (Figure 7-4A & B) appeared especially responsive to flow perturbations, and this was reflected in the variation in load (Figure 7-4C).

The concentration of indicator bacteria in freezing works

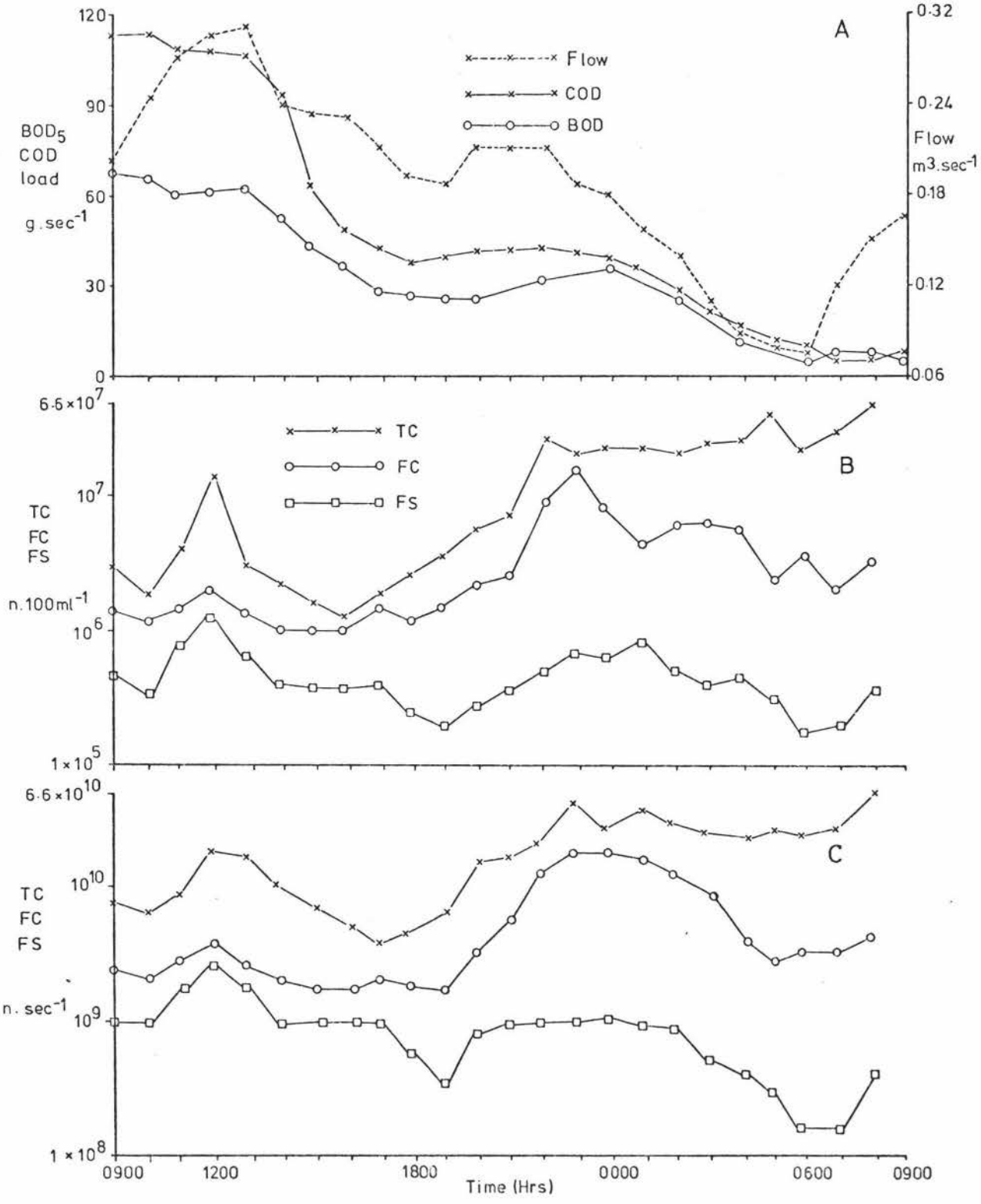


Figure 7-3 Variation in BOD_5 and COD load (A), TC, FC, and FS concentration (B), and TC, FC, and FS load (C) in sewage effluent sampled on 23rd June, 1976.

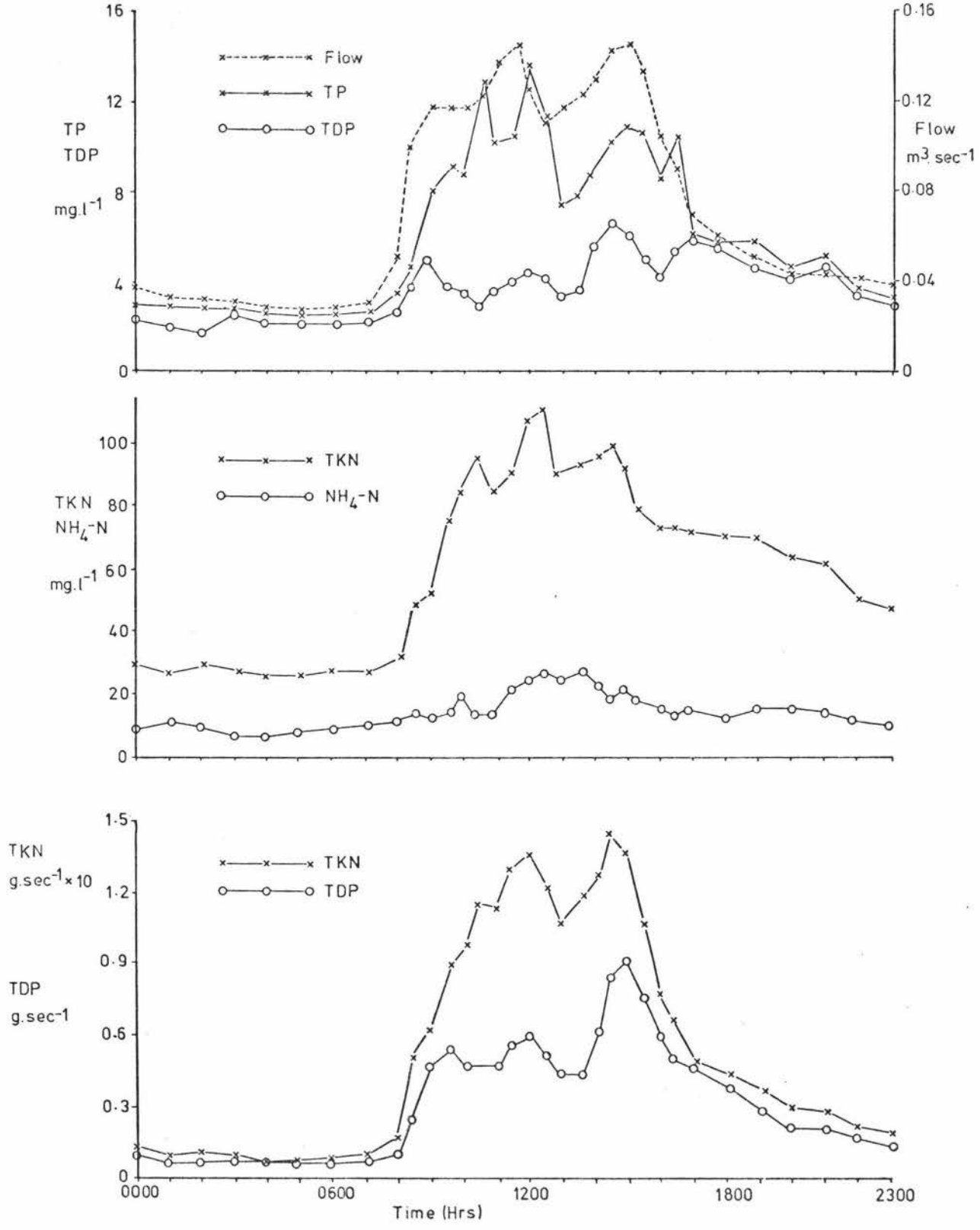


Figure 7-4 Variation in TP and TDP concentration (A), TKN and NH_4-N concentration (B), and TDP and TKN load (C), in freezing works effluent sampled on 24 March, 1976.

effluent appeared only weakly related to flow (Figure 7-5). FC concentration and load increased during killing hours and decreased overnight (Figure 7-5A & B). TC concentration increased during the non-killing period (Figure 7-5A) and appeared to be inversely related to flow. This may have been due to a proliferation of non-faecal coliform numbers, which were also the only bacterial group studied, to increase on incubation under laboratory conditions (Chapter 5). The concentration of total coliform bacteria appeared to be the dominant factor affecting load (Figure 7-5B), whereas both concentration and flow equally affected the load of faecal coliform bacteria.

The concentration of chemical species in soil-treated dairy shed effluent showed an obvious relationship to the disposal times of the raw effluent. Peaks in concentration of N and P forms (Figure 7-6A & B) did not coincide with peak flow rate but preceded or succeded it by periods of up to 1½ hours. Some chemical species, such as DIP and NO_3-N, which formed only a small proportion of the total P and N respectively, showed small fluctuations which could be ascribed to a milking event (Figure 7-6A).

The time difference between peak flow and peak concentration was even more pronounced with coliform bacteria. In the winter sampling event (Figure 7-7A), both total and faecal coliforms reached a peak in concentration approximately 2½ hours after the afternoon milking event peak in flow.

The concentration of the bacteria was the main factor determining load (Figure 7-7B) as the loading and concentration curves were virtually identical in shape.

In the early summer sampling event, the application of effluent from the afternoon milking event had little effect on total coliform concentrations but caused a small peak in faecal coliform concentration approximately 1 hour after the peak flow (Figure 7-7C). Application of effluent the following morning, however, caused a large peak in both total and faecal coliform concentrations, which preceded the peak in flow by 1 hour.

Thus it can be seen from the above results that while peaks in concentration and load of the various parameters are related to the degree of activity generating the effluent, the relationship is not

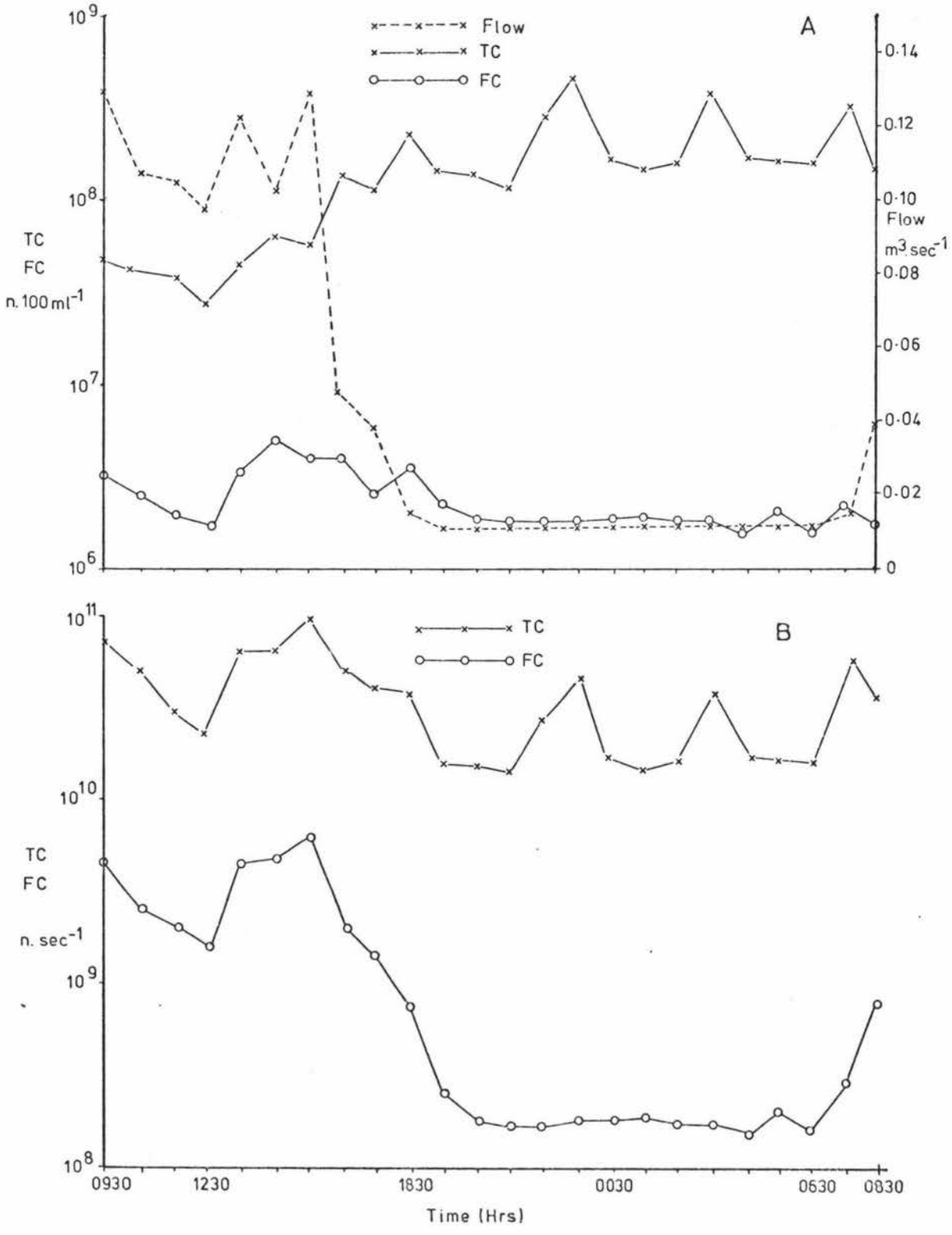


Figure 7-5 Variation in TC and FC concentration (A), and load (B) in freezing works effluent sampled on 11 - 12 November, 1976.

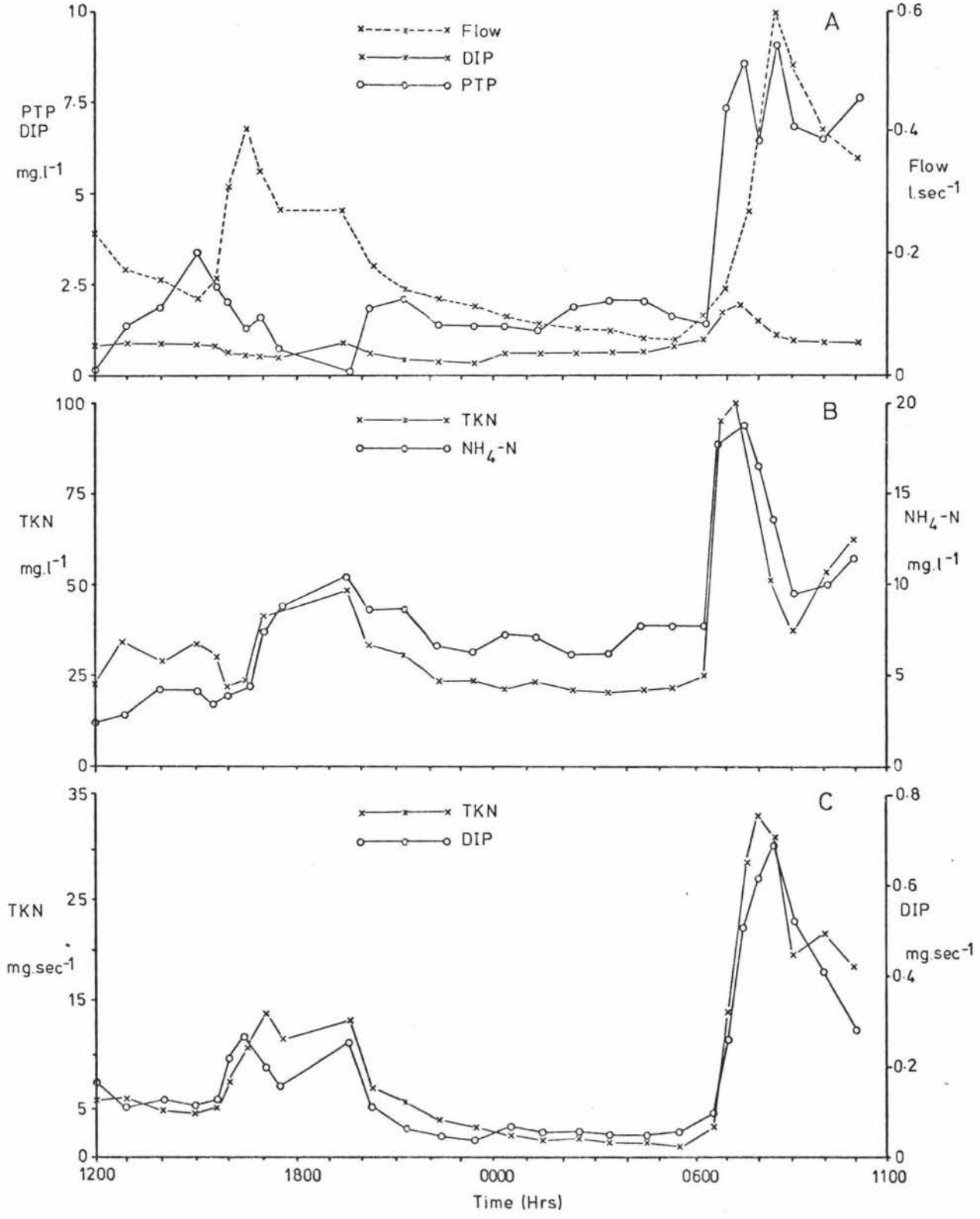


Figure 7-6 Variation in DIP and PTP concentration (A), TKN and NH_4-N concentration (B), and DIP and TKN load (C) in dairy shed effluent sampled on 29-30 November, 1976.

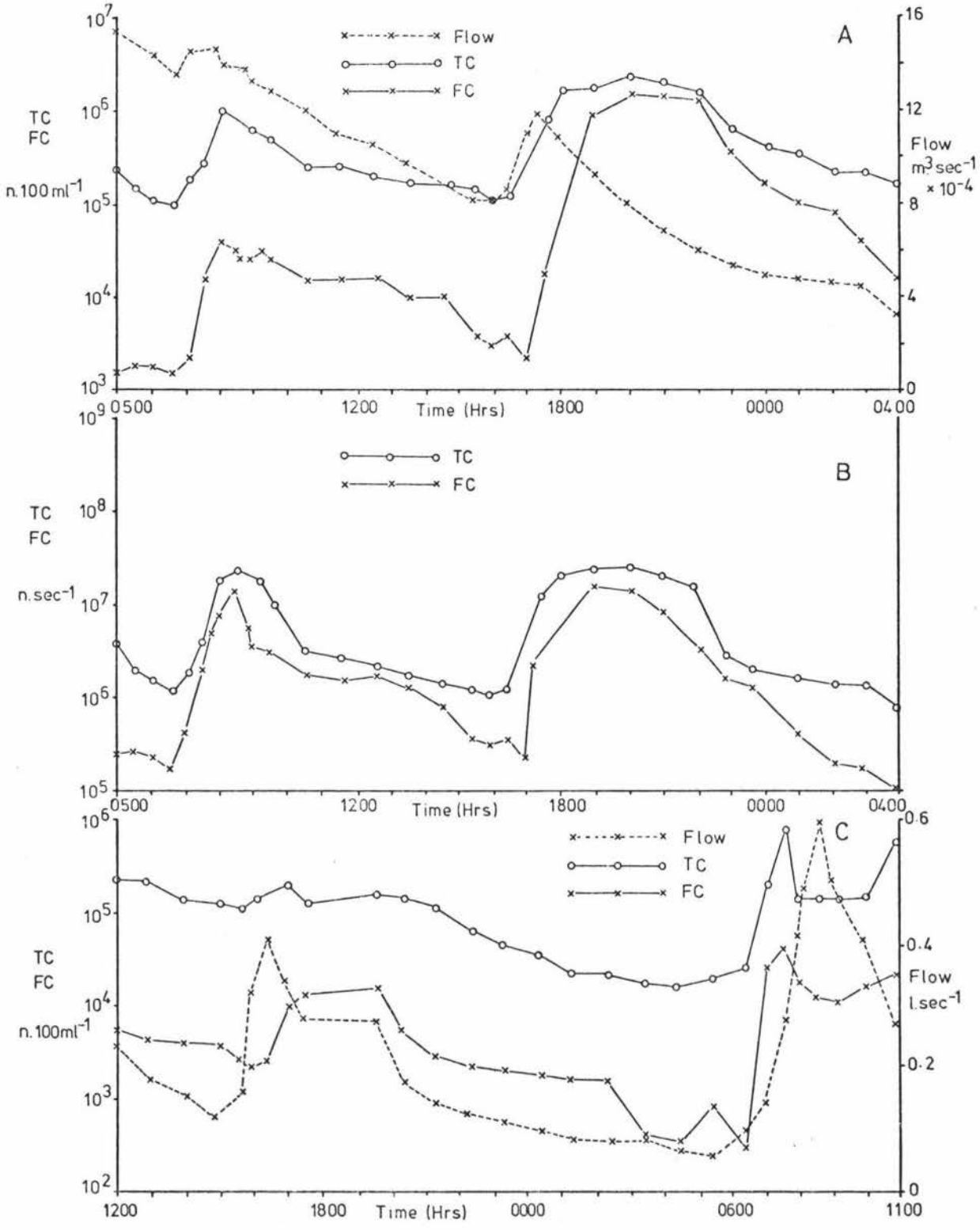


Figure 7-7 Variation in TC and FC concentration (A and C) and load (B) in dairy shed effluent sampled on 16-17 September (A and B) and 29-30 November (C), 1976.

clear-cut. From the point of view of formulating a sampling policy for these effluents, it would be dangerous to sample on the basis of regular diurnal flow patterns, without further investigation of the relationship of the flow to the parameter concerned.

7.4.2 Determination of sampling frequency

1. Computer integration for estimation of loads

I. Sewage effluent

The 10% limit on variation of load was first exceeded on the use of a theoretical 4-hourly sampling interval (Table 7-4). This variation occurred with only two nutrient forms (NO_3-N and TPN), both of which are only minor constituents of the TN in sewage effluent (see Chapter 6). Using an 8-hour sampling interval, DOP also exceeded 10% variation of the 'actual' load. At 12-hour intervals, most other parameters had load variations greater than 10% of that estimated using all samples. On the basis of this sampling event it could be recommended that a 2-hourly sampling interval be used to estimate loads of all nutrient forms, 4 hourly if insignificant forms such as NO_3-N and TPN can be ignored, and 8 hourly to ensure at least an accurate estimate of total forms (TN, TP, and TS).

II. Freezing works effluent

PTP was the first nutrient form to exceed 10% variation of load (Table 7-5) and this occurred using a 2-hourly sampling interval. As PTP accounted for almost 50% of the TP in the freezing works effluent this error cannot be ignored and a sampling interval of 1 hour is recommended. The variation in particulate forms was associated with works activity, as discussed in the previous section. It is interesting to note that TPN also exhibited high variation. To ensure an estimate within 10% of the true load of TN and TP a sampling interval of 2 hours should be used.

III. Dairy shed effluent

Results from the winter sampling run (Table 7-6) indicated that a one-hourly sampling interval was necessary to characterise N and P form loads to within 10%. The use of 2-hourly sampling intervals was sufficient to estimate adequately loads in the early summer sampling run (Table 7-7). This result was contrary to that expected from an examination of the hydrographs. It was thought that more frequent sampling would be needed to estimate accurately the loads contributed

Table 7-4 Total load of N and P forms and total solids leaving Palmerston North Sewage Treatment Plant from 9.11.76 and 11.11.76 as estimated using various sampling intervals.

Parameter	As collected 60 samples	Hourly 49 samples	Variance %	2 hourly odd (1100-1300-1500 ...) 25 samples	Variance %	2 hourly even (1200-1400-1600 ...) 24 samples	Variance %	4 hourly odd (1100-1500-1900 ...) 13 samples	Variance %	4 hourly even (1200-1600-2000 ...) 12 samples	Variance %	8 hourly odd (1100-1900-0300 ...) 7 samples	Variance %	8 hourly even (1200-2000-0400 ...) 6 samples	Variance %	12 hourly 1200-0000 4 samples	Variance %	12 hourly 1800-0600 4 samples	Variance %	24 hourly 1200 2 samples	Variance %	24 hourly 0000 2 samples	Variance %
DIP	69.83	69.78	-0.1	70.15	+0.5	69.93	+0.1	70.29	+0.7	68.09	-2.5	69.03	-1.1	70.55	+1.0	80.99	+16.0	78.83	+12.9	97.93	+40.2	67.19	-3.8
DOP	23.36	23.18	-0.5	23.72	+1.8	23.72	+1.8	22.49	-3.5	25.19	+6.1	15.26	-34.5	19.73	-15.3	22.94	-1.5	19.66	-15.6	31.80	+36.5	20.45	-12.2
TDP	92.13	92.95	-0.2	93.87	+0.8	93.65	+0.6	92.78	-0.4	93.28	+0.2	84.29	-9.5	90.29	-3.0	103.93	+11.6	101.13	+8.6	129.74	+39.3	87.65	-5.9
PTP	29.40	29.45	+0.2	28.50	-3.1	28.43	-3.3	27.27	-7.2	26.95	-8.3	25.22	-0.6	26.78	-2.1	26.15	-11.1	23.52	-20.0	23.56	-19.9	21.87	-25.6
TP	122.53	122.41	-0.1	122.37	-0.1	122.08	-0.4	120.06	-2.0	120.23	-1.9	113.52	-7.4	119.06	-2.8	130.08	+6.2	142.01	+15.9	153.30	+25.1	109.52	-10.6
DAN	802.70	802.2	-0.2	787.9	-2.0	785.9	-2.2	773.9	-3.7	805.6	+0.2	778.5	-3.1	862.15	+7.3	958.8	+19.3	974.1	+21.2	1127.2	+40.2	859.8	+7.0
NO_3-N	22.3	22.4	+0.4	22.2	-0.4	22.2	-0.4	22.3	0	16.7	-25.1	26.4	+18.4	17.0	-23.7	19.8	-21.2	23.8	+6.9	26.6	+19.3	13.4	-39.9
NH_4-N	525.6	524.4	-0.2	520.1	-1.0	518.4	-1.4	500.2	-4.8	514.4	-2.1	502.4	-4.4	558.9	+6.3	622.8	+18.5	661.2	+25.8	731.5	+39.2	543.5	+3.4
TKN	826.0	825.2	-0.1	810.1	-1.9	808.1	-2.2	796.2	-3.6	822.3	-0.4	804.9	-2.6	879.1	+6.4	978.6	+18.5	1053.9	+27.6	1153.8	+39.7	873.2	+5.7
TVN	694.7	693.5	-0.1	873.8	-2.3	871.0	-2.6	858.1	-4.1	897.6	+0.3	882.1	-1.4	965.4	+7.9	1062.3	+21.0	1101.4	+23.1	1420.6	+58.8	878.2	-1.8
TN	917.0	916.0	-0.1	896.0	-2.3	893.2	-2.6	880.5	-4.0	914.5	-0.3	906.5	-0.9	982.4	+7.1	1102.1	+20.2	1172.8	+27.9	1447.3	+57.8	891.7	-2.8
TPN	91.0	91.2	+0.2	86.8	-4.6	85.8	-5.7	85.0	-6.6	111.2	+22.2	103.7	+13.9	103.2	+13.4	123.5	+35.7	134.9	+48.3	293.4	+222.4	18.5	-79.7
TS	7963	7820	-2.0	8075	+1.0	8068	+1.3	7999	+0.4	7269	-8.7	8643	+8.5	8203	+3.0	7507	-5.7	8672	+8.9	2526	-68.3	10562	+32.6

Table 7-5 Total load of N and P forms leaving effluent treatment plant of Longburn Freezing Co. Ltd. from 23.3.76 1000 to 26.3.76 1000 as estimated using various sampling intervals.

Parameter (kg)	As collected 98 samples	1 hourly 73 samples	Variance %	2 hourly starting 1000 37 samples	Variance %	2 hourly starting 1100 36 samples	Variance %	4 hourly starting 1000 19 samples	Variance %	4 hourly starting 1100 18 samples	Variance %	8 hourly starting 1000 10 samples	Variance %	8 hourly starting 1100 9 samples	Variance %	12 hourly 1100-2300 6 samples	Variance %	12 hourly 0600-1800 6 samples	Variance %	24 hourly 1200 3 samples	Variance %	24 hourly 1800 3 samples	Variance %
DIP	61.79	61.23	-0.9	59.42	-3.8	62.69	+1.4	56.02	-9.3	60.69	-1.8	55.4	-10.4	50.48	-18.3	53.5	-13.4	54.7	-11.5	62.18	+0.6	77.58	+25.5
DOP	7.19	7.37	+2.5	7.74	+7.6	7.12	-0.8	8.62	+19.9	7.28	+1.2	9.77	+35.9	8.00	+11.3	3.90	-45.7	8.24	+14.6	5.38	-25.2	6.59	-8.3
TDP	68.98	68.61	-0.5	67.16	-2.6	69.81	+1.20	64.65	-6.3	67.98	-1.4	65.20	-5.5	58.48	-15.2	57.40	-16.8	62.95	-8.7	67.56	-2.1	84.17	+22.0
PTP	57.26	56.84	-0.7	62.79	+9.6	48.85	-14.7	42.36	-26.0	48.81	-14.8	37.04	-35.3	46.98	-18.0	46.36	-17.0	14.19	-75.2	116.25	+103.0	24.18	-57.8
TP	126.24	125.45	-0.6	129.95	+2.9	118.66	-6.0	107.02	-15.2	116.80	-7.5	102.24	-17.0	105.40	-16.5	103.80	-17.8	77.14	-38.9	183.80	+45.6	108.36	-14.2
DKN	868.0	842.6	-2.9	852.8	-1.8	817.7	-5.8	844.3	-2.7	765.6	-11.8	794.2	-8.5	721.0	-16.9	758.5	-12.6	551.1	-36.5	1295.6	+49.2	835.1	-3.8
NO_3-N	5.2	5.0	-3.8	5.3	+3.3	4.6	-11.5	5.4	+3.8	4.9	-5.8	5.2	0	4.5	-13.5	4.0	-23.1	6.1	+17.3	5.3	+1.9	7.3	40.4
NH_4-N	284.5	282.1	-0.8	285.4	+0.3	278.2	-2.2	276.4	-2.9	256.6	-9.8	229.1	-19.5	235.4	-17.3	239.6	-15.8	165.8	-41.7	436.1	+53.2	189.6	-33.4
TDN	873.2	847.6	-2.9	858.1	-1.7	822.5	-5.8	849.7	-2.7	770.6	-11.7	799.3	-8.5	725.5	-17.0	762.6	-12.7	557.2	-36.2	1300.9	+49.0	842.4	-3.5
TKN	1216.9	1201.7	-1.3	1202.8	-1.2	1188.0	-2.4	1131.1	-7.1	1148.6	-5.6	1065.5	-12.4	1068.0	-12.2	1072.6	-11.9	807.1	-33.7	1884.5	+54.9	1154.8	-5.1
TN	1222.1	1206.7	-1.3	1208.2	-1.1	1192.6	-2.3	1136.5	-6.9	1153.6	-5.5	1070.7	-12.3	1072.0	-12.2	1076.6	-11.8	813.2	-33.4	1889.8	+54.8	1162.1	-4.8
TPN	348.9	359.0	+2.9	350.0	+0.3	370.3	+6.1	286.8	-17.8	383.0	+9.8	271.3	-12.3	346.9	-0.6	314.0	-10.0	256.0	-26.6	588.9	+68.8	319.7	-8.4

Table 7-6 Total load of solids, COD, and N and P forms leaving the weir, No.4 Dairy Unit, between 16.9.76 0500 and 17.9.76 0500 as estimated using various sampling intervals.

Parameter (g)	As collected 38 samples	Hourly 25 samples	Variance %	2 hourly starting 0500 13 samples	Variance %	2 hourly starting 0600 12 samples	Variance %	4 hourly starting 0500 7 samples	Variance %	4 hourly starting 0600 6 samples	Variance %	8 hourly starting 0500 4 samples	Variance %	8 hourly starting 0600 3 samples	Variance %	12 hourly 1200-0000 2 samples	Variance %	12 hourly 0600-1800 2 samples	Variance %	24 hourly 1200 1 sample	Variance %	24 hourly 0000 1 sample	Variance %
DIP	13.47	13.96	+3.7	14.12	+4.7	13.59	+1.0	12.89	-4.3	9.45	-29.8	12.33	-8.5	10.68	-20.7	14.13	+4.9	10.37	-23.0	16.09	+19.4	10.00	-25.7
DOP	23.61	25.91	+9.7	22.72	-3.8	28.98	+22.7	18.47	-21.8	25.74	+9.0	10.68	-54.8	18.79	-20.4	14.65	-37.9	50.47	+113.8	15.31	-35.2	13.28	-43.8
TDP	37.08	39.87	+4.8	36.84	-0.7	42.47	+14.5	31.36	-15.4	35.20	-5.1	23.01	-37.9	29.47	-20.5	28.79	-22.4	60.84	+64.1	31.40	-15.3	23.28	-37.2
PTP	57.73	52.97	-8.8	60.50	+4.8	44.71	-22.6	46.18	-20.0	39.19	-32.1	51.80	-10.3	29.44	-49.0	68.32	+18.3	54.68	-5.3	42.41	-26.5	123.16	+113.3
TP	94.81	92.84	-2.1	97.34	+2.6	87.18	-8.1	77.52	-18.2	74.39	-21.5	74.81	-21.1	58.90	-37.9	97.11	+2.4	112.52	+18.6	73.81	-22.1	146.44	+38.8
DKN	785.0	780.5	-0.6	747.4	-4.8	798.2	+1.7	717.9	-8.5	680.8	-13.3	635.8	-19.0	559.0	-28.8	824.4	+7.3	1001.3	+27.6	917.6	+16.9	683.3	-13.0
NO_3-N	199.1	199.7	+0.3	198.4	-0.3	200.2	+0.6	196.3	-1.4	205.1	+3.2	191.9	-3.6	223.4	+12.2	182.7	-8.2	191.4	-3.9	182.8	-8.2	182.7	-8.2
NH_4-N	216.6	216.5	-0.1	204.1	-5.8	227.0	+4.8	191.8	-11.4	211.2	-2.5	205.7	-5.0	184.0	-15.1	221.8	+2.4	300.8	+38.9	188.2	-13.1	292.9	+35.2
TDN	984.1	980.1	-0.6	945.8	-3.9	998.4	+1.5	914.2	-7.1	885.9	-10.0	827.8	-15.9	782.4	-20.5	1025.2	+4.1	1192.7	+21.2	1100.4	+11.8	866.1	-12.00
TKN	1002.7	1008.7	+0.6	953.6	-4.9	1042.4	+4.0	897.9	-10.5	891.1	-11.1	806.9	-19.5	704.2	-29.7	987.0	-1.6	1395.0	+39.1	1062.1	+5.9	827.8	-17.4
TN	1201.8	1208.3	+0.5	1142.0	-4.1	1242.7	+3.4	1094.2	-9.0	1096.2	-8.8	998.8	-16.9	927.7	-22.8	1169.7	-2.7	1586.4	+32.0	1244.8	+3.5	1010.6	-15.9
TPN	217.7	228.2	+4.8	206.2	-5.3	244.2	+12.2	180.0	-17.3	210.3	-3.4	171.0	-21.5	145.3	-33.3	144.5	-33.6	393.7	+80.8	144.5	-33.6	144.5	-33.6
TS	11232	11081	-1.3	11095	-1.2	10740	-4.4	10687	-4.9	10614	-5.5	9518	-15.3	9350	-16.8	9034	-19.7	16409	+46.1	7028	-37.4	13276	+18.2
COD	8277	8458	+2.1	7902	-4.5	8891	+7.4	7862	-4.8	7788	-5.9	7149	-13.6	7049	-14.8	8021	-3.1	10048	+21.4	8122	-1.8	7810	-5.6

Table 7-7 Total load of solids and N and P forms leaving the weir, No.4 Dairy Unit, between 29.11.76 1200 and 30.11.76 1200 as estimated using various sampling intervals.

Parameter (g)	As collected 29 samples	Hourly 25 samples	Variance %	2 hourly starting 1200 13 samples	Variance %	2 hourly starting 1300 12 samples	Variance %	4 hourly starting 1200 7 samples	Variance %	4 hourly starting 1300 6 samples	Variance %	8 hourly starting 1200 4 samples	Variance %	8 hourly starting 1300 3 samples	Variance %	12 hourly 1200-0000 3 samples	Variance %	12 hourly 1800-0600 2 samples	Variance %	24 hourly 1200 2 samples	Variance %	24 hourly 0000 1 sample	Variance %
DIP	16.10	16.16	+0.3	16.81	+4.4	16.48	+2.4	17.26	+7.2	14.47	-10.1	14.65	-9.0	14.10	-12.4	15.03	-6.6	14.88	-7.6	18.64	+15.8	11.52	-28.4
DOP	21.89	21.56	-1.5	23.95	+9.4	22.41	+2.4	21.73	-0.7	14.85	-32.2	23.51	+7.5	20.28	-7.4	24.50	+11.9	28.16	+28.6	34.31	+56.7	14.51	-33.7
TDP	37.99	37.73	-0.7	40.76	+7.3	38.90	+2.4	39.99	+5.2	29.32	-22.8	38.17	+0.4	34.39	-9.5	39.53	+4.1	43.04	+13.3	52.95	+39.4	26.03	-31.5
PTP	69.44	73.93	+6.5	64.25	-7.5	75.03	+8.1	67.16	-3.3	62.21	-10.4	51.76	-25.5	35.82	-48.4	46.64	-32.9	21.33	-69.3	70.34	+1.3	24.31	-65.0
TP	103.86	106.86	+2.9	98.06	-5.6	107.98	+4.0	106.15	+2.2	91.53	-11.9	89.23	-14.1	70.21	-32.4	86.17	-17.0	64.37	-38.0	123.29	+18.70	50.34	-51.5
DKN	463.2	463.4	+0.1	444.2	-4.1	468.8	+1.2	481.6	+3.9	395.1	-14.7	386.4	-16.6	311.3	-32.8	383.5	-17.2	418.1	-9.7	455.8	-1.6	315.4	-31.9
NO_3-N	31.9	32.1	+0.7	30.3	-4.9	31.7	-0.6	33.2	+4.1	30.3	-5.1	25.8	-19.1	18.0	-43.6	22.5	-29.5	19.8	-37.9	34.3	+7.2	10.8	-66.1
NH_4-N	163.2	165.8	+1.6	159.2	-2.5	167.2	+2.4	166.5	+2.0	142.8	-12.5	143.2	-12.3	126.3	-22.6	129.4	-20.7	151.1	-7.4	123.9	-24.1	136.6	-16.3
TDN	495.0	495.5	+0.1	474.5	-4.1	500.5	+1.1	514.8	+4.0	425.3	-14.1	412.2	-16.7	329.2	-33.5	405.9	-18.0	437.8	-11.6	490.1	-0.9	326.2	-34.1
TKN	791.3	803.4	+1.5	792.2	+0.1	824.1	+4.1	844.6	+6.7	614.7	-22.3	690.8	-12.3	495.9	-37.3	682.2	-13.8	582.4	-26.4	928.1	+17.3	446.7	-43.5
TN	823.2	835.5	+1.5	822.6	-0.1	855.8	+4.0	877.8	+6.6	644.9	-21.6	716.6	-12.9	513.9	-37.6	704.6	-14.4	602.2	-26.8	962.4	+16.9	457.5	-44.4
TPN	328.2	340.0	+3.4	348.1	+6.1	355.2	+8.2	362.9	+10.6	219.6	-43.1	304.3	-7.3	184.6	-43.8	298.7	-9.0	164.4	-49.9	472.3	+43.9	131.3	-60.0
TS	14331	14143	-1.3	14548	+1.5	14273	-0.4	15305	+6.8	12007	-14.2	14096	-1.6	10066	-29.7	15571	+8.6	11823	-17.5	17322	+20.9	13857	-3.3

by the sharp peaks associated with summer effluent application. The lag in concentration and load after effluent application (section 7.4.1), however, may account for the apparent longer sampling interval needed. The influence of rainfall/runoff by causing contributions to the total load from the soil itself, may have accounted for the 1-hour sampling interval needed.

Obviously the soil-moisture condition of the disposal site must affect the sampling frequency needed if only the contributions of the dairy shed effluent itself are considered. The dryer the soil the better the site becomes for disposal of effluent because the effluent will diffuse through the soil horizontally as well as vertically. Contributions to the total load of 'treated' effluent will reach the weir over a longer time period, thus allowing longer sampling intervals. When the soil is saturated, however, the sprayed effluent passes through to the tile drains in a manner akin to mass flow, so that shorter sampling intervals will be needed to characterise the total load.

2. Use of statistical distributions to determine summary measures of effluent quality

Probability plots of nutrient parameters (Figure 7-8) suggested that concentration values determined from each of the three effluent sources were members of a normal distribution. The usual use of such plots is for defining a long-term distribution (e.g., annual) on the basis of results obtained from samples taken on a random, or, as is more usual, a systematic basis (Montgomery and Hart, 1974). The results presented, however, suggest that they may also be useful for characterising the distribution of concentrations (or loads) from an effluent source on a short-term, fixed-time basis.

The forms of N and P (as typified by TP and TKN in this example) in sewage and freezing works effluents showed a good fit to a normal distribution whereas for the winter sampling run of dairy shed effluent, the curve of the data (Figure 7-8A) suggests that a fit to this distribution was not obtained. There are two possible explanations for this lack of fit. Either insufficient data were obtained to describe the distribution adequately or, the time based nature of the distribution has been upset by the effect of runoff waters (Figure 7-1). This latter possibility would seem the more likely as during a sampling period unaffected by runoff, a better

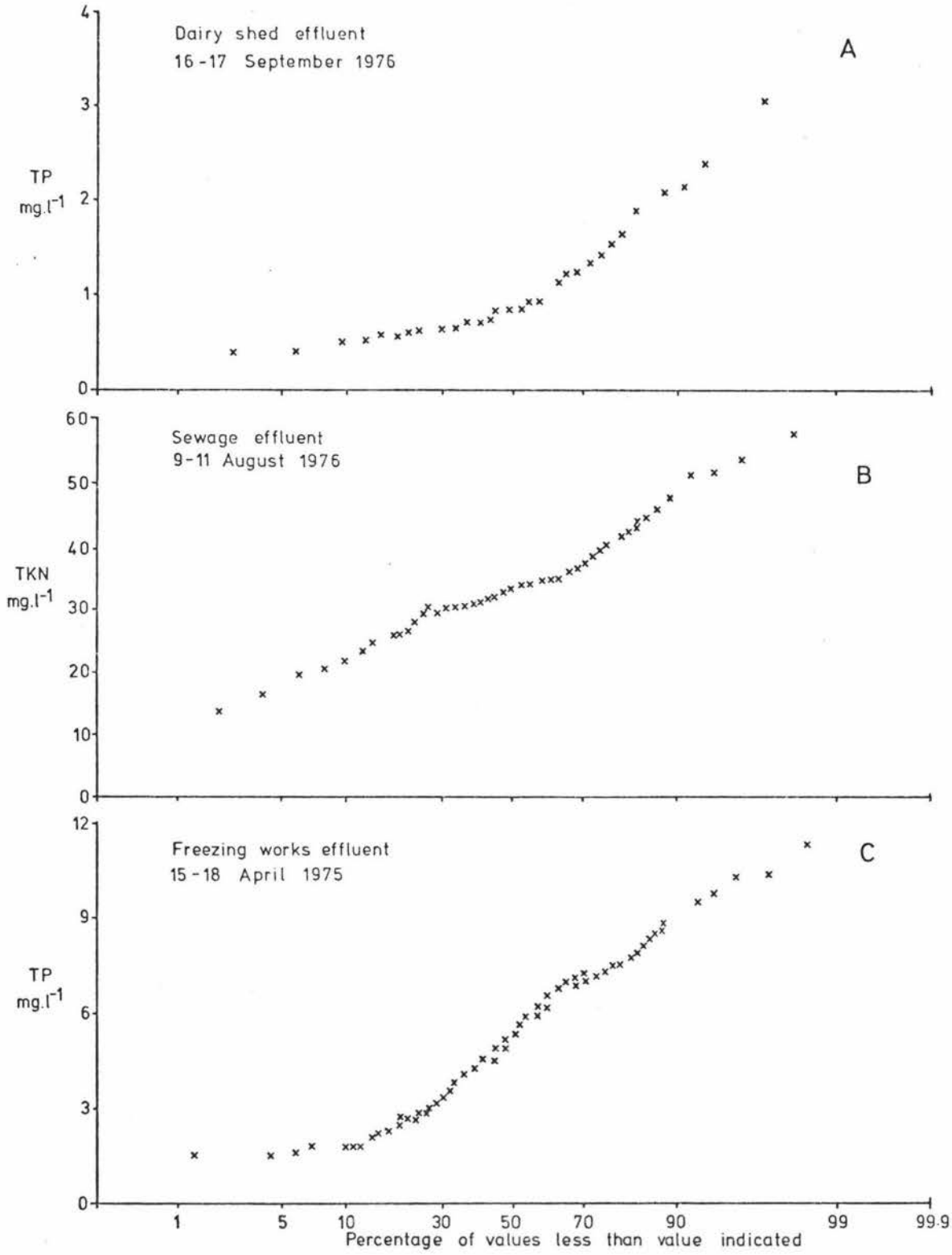


Figure 7-8 Probability plots of TP (A), TKN (B), and TP(C) concentrations from dairy shed, municipal sewage, and freezing works effluent sampling runs, respectively.

fit of data was obtained.

The oxygen-demanding parameters of dairy shed effluent are described adequately by either a normal or a log-normal distribution (Figure 7-9A), although a slightly better fit is obtained with a normal distribution. In the case of sewage effluent, however, a far better fit was obtained with the log-normal distribution. Insufficient oxygen-damand data for freezing works effluent were available for any one run, however the excellent log-normal fit for total solids concentration suggests that BOD_5 and COD would be similar, as the solids fraction of freezing works effluent is high in oxygen demanding organics (Bond and Straub, 1974).

Bacterial indices are best described using the log-normal distribution (Figure 7-10). The poor fit obtained with $\log_{10}$TC in the sewage effluent was probably due to insufficient data being taken to describe the distribution. It should be emphasized, however, that these distributions are empirical in nature and therefore some variation from the desired distribution is to be expected. Empirical adjustments such as those used by Montgomery and Hart (1974) may improve the fit of the distribution.

The sampling frequencies needed to characterise the arithmetic mean with a precision of 10% of the total range for the distributions described in Figures 7-8 to 7-10 are given in Table 7-8. Values of $\underline{N}$ for the median concentration are simply 1.25 times that for the arithmetic mean in the case of a normal distribution (Table 7-3). For a log-normal distribution the values of $\underline{N}$ for mean and median will be the same if $\bar{x}$ occurred on the 50 percentile value, and $\underline{N}$ (median) will be less than $\underline{N}$ (mean) if $\bar{x}$ occurred either side of this percentile value.

The importance of the choice of precision cannot be over emphasized. p is related inversely to $\sqrt{\underline{N}}$ and hence any reduction in p (improvement in precision) will result in a large increase in the number of samples needed to attain that precision. For example, the number of samples needed to define the arithmetic mean TKN concentration in sewage effluent during 2 days of sampling, with a precision of 4.4 $mg.\ell^{-1}$ (10% of the range) at the 95% confidence level is 20. The number of samples needed to define the same statistic with a precision of 2 $mg.\ell^{-1}$ and 1 $mg.\ell^{-1}$ is 101 and 404,

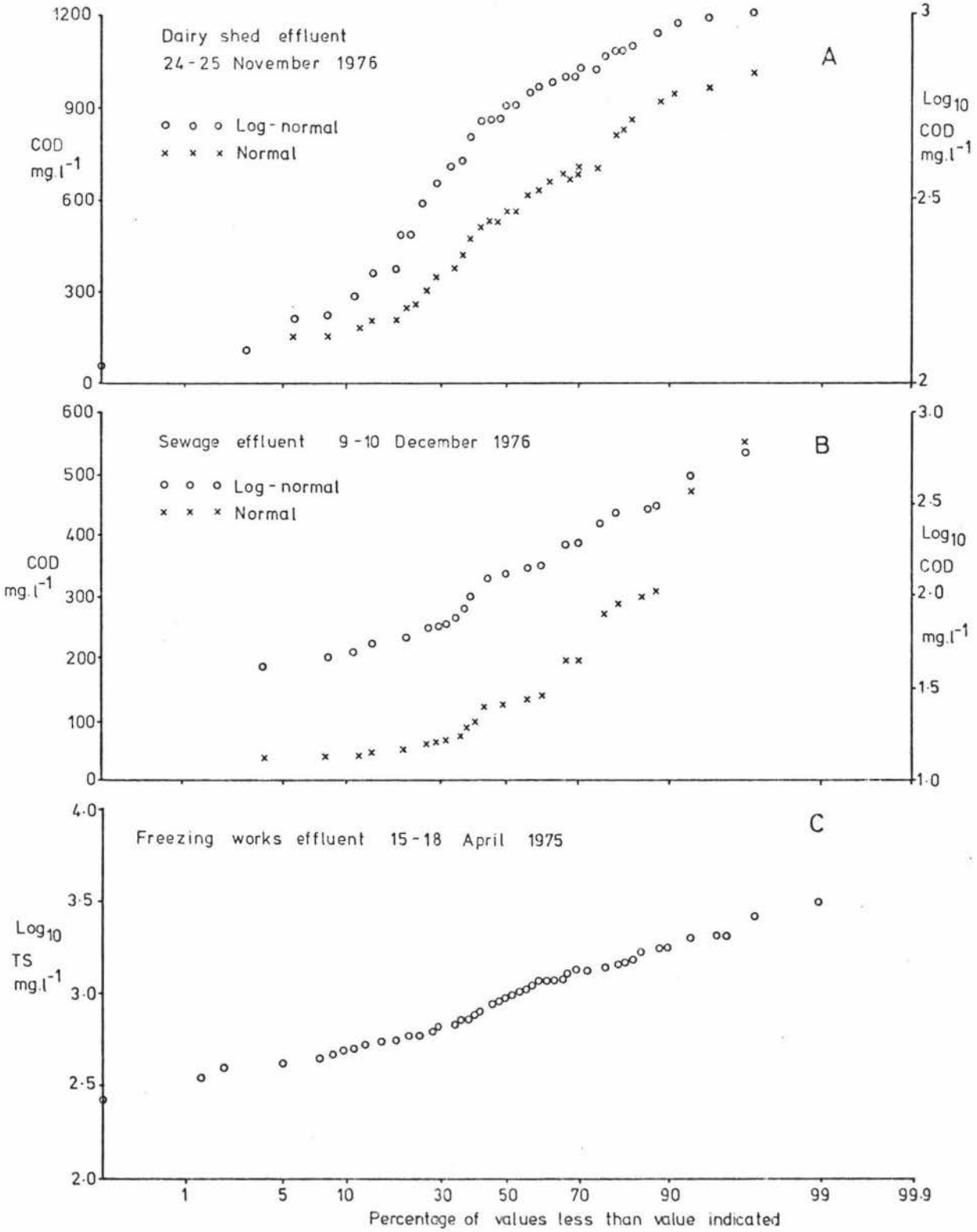


Figure 7-9 Probability plots of COD (A and B) and TS concentrations from dairy shed, municipal sewage, and freezing works effluent sampling runs, respectively.

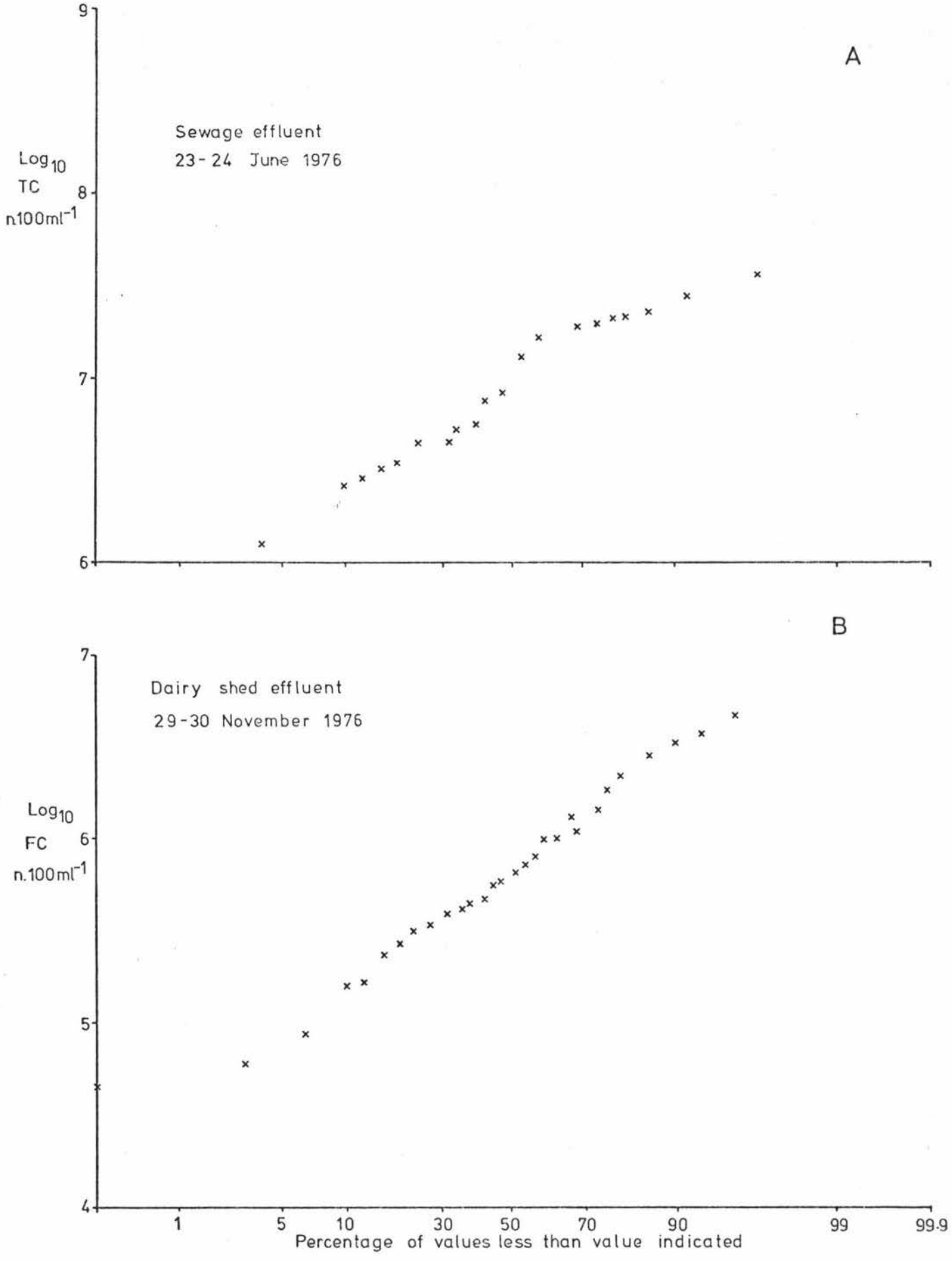


Figure 7-10 Probability plots of TC (A) and FC (B) concentrations from municipal sewage and dairy shed effluent sampling runs, respectively.

Table 7-8 Estimated sampling frequencies needed to characterise the arithmetic mean concentration of chemical and microbiological parameters in effluent discharges.

Effluent	Ref.date	Duration(hr)	Figure	Parameter	Distribution	95% Confidence level		90% Confidence level	
						N	frequency(hr)	N	frequency(hr)
Dairy shed	16/9/76	24	7-8	TP	Normal?	26	0.92	18	1.33
Sewage	9/11/76	48	7-8	TKN	Normal	20	2.40	14	3.42
Freezing works	15/4/75	72	7-8	TP	Normal	25	2.88	18	4.00
Dairy shed	24/11/76	24	7-9	COD	Normal	36	0.66	26	0.92
Sewage	9/12/76	30	7-9	COD	Log-normal	34	0.88	24	1.25
Freezing works	15/4/75	72	7-9	TS	Log-normal	22	3.27	15	4.80
Sewage	23/6/76	24	7-10	TC	Log-normal	112	0.21	78	0.30
Dairy shed	29/11/76	24	7-10	FC	Log-normal	54	0.44	38	0.63

Where N = the number of systematic samples needed in stated duration.

respectively. Alternatively, a lowering of the confidence level at which the statistic is acceptable will decrease the number of samples necessary. In the above example, using the original precision $\underline{N}$ = 14, 9, and 3 for the 90, 80, and 50% confidence levels, respectively. The method can also be used to calculate the precision of already collected data by rearrangement of the equation. For the arithmetic mean of normally distributed data $p = \frac{k\hat{\sigma}}{\sqrt{\underline{N}}}$. For the example discussed above where $\underline{N}$ = 60 (Figure 7-8), $p = 2.60$ mg.ℓ^{-1}.

The number of samples ($\underline{N}$) defined in the above procedure refers to randomly-sampled data. The number of samples actually needed may be somewhat less than this as systematic sampling was used (Rainwater and Avrett, 1962; Montgomery and Hart, 1974). At the moment, no methematically proven procedure exists for quantifying this difference.

7.5 General Discussion

The suggestion that representativeness is only obtained by random sampling (Mancy and Weber, 1971) would not appear to be valid in the sampling of effluents. For the effluents under consideration it would be impossible to take 2 random samples during any 24-hour period; that is 2 samples which are completely unrelated or unaffected by the other sample. From the results and discussion presented, it can be seen that each and every sample taken is a reflection of the processes which have taken place to generate that sample. These processes are persistent and cyclical in nature and hence systematic sampling will give a more precise description of the effluent than a similar number of random samples, providing that the sampling interval is short enough to reveal the persistence.

It is perhaps fortuitous from a sampling point of view that most of the flow of all the effluents, but especially dairy shed and freezing works effluents, occurs during daylight hours. This is also the period of the greatest variability in concentration and load. To obtain better definition of mean concentration, mean load, total load, or other summary values more frequent systematic sampling should take place during this period. Some mathematical rationalisation of this theory is clearly needed.

In conclusion, it may be stated that the overall design of a sampling programme should be governed by the information which the programme is intended to obtain. A rationalisation of local resources is a necessary corollary to this statement. There is no use, for example, in collecting 50 samples per day if only 5 of them can be meaningfully analysed. Thus, regulatory agencies such as Regional Water Boards may be justified in obtaining a loading assessment of a particular effluent which is within 100% of the actual daily load by sampling only 2 or 3 times in 24 hours. Such data may be useful for establishing a broad regional assessment of total effluent loads. It is important, however, that there be an awareness of the likely errors caused by this infrequent sampling.

Detailed sampling on different effluent types by research organisations will therefore be necessary to establish guidelines for the Regional Water Boards, as clearly the number of samples

necessary to establish an assessment of load to within any prescribed degree of accuracy varies markedly between different effluent types.

SUMMARY

The work presented in this thesis may be summarised as follows:

1. Answers to a questionnaire directed to Regional Water Board staff indicated that little emphasis is being placed on effluent compliance monitoring in Regional Water Boards. This appeared to be due to:
   (i) Some misunderstanding by Boards of the need to set and enforce effluent standards in order to maintain receiving water standards.
   (ii) Lack of resources to set up and maintain an effluent compliance monitoring programme, and
   (iii) Lack of technical guidelines to run such a programme.

2. Little work has been done on preservation of N and P forms in effluent samples. Results of an experiment on the preservation of N and P forms in samples of municipal sewage and freezing works effluents indicated that the best overall preservative treatments are; freezing and refrigeration for filtered and unfiltered samples, respectively, with the addition of 50 mg $HgCl_2 \ell^{-1}$ of effluent prior to such storage greatly assisting preservation in freezing works samples.

3. Experiments on growth/die-off characteristics of indicator bacteria in samples of municipal sewage, freezing works, and dairy shed effluents at room temperature and 4C, indicated that these samples could be held for up to 6-9 hours at 4C before appreciable changes in bacterial populations became apparent. In the effluent samples studied, both faecal coliforms and faecal streptococci showed the most rapid increase in dairy shed effluent held at room temperature. The results of the experiment demonstrated that care must be taken in the use of the FC:FS ratio in effluent samples at it may change with time.

4. A review of the literature indicated that little work had been done on the characterisation of N and P forms in effluent samples. Monitoring studies showed that in the sewage effluent approximately 60% of both TP and TN was in dissolved inorganic form, slightly less and slightly greater than 20% in dissolved organic and particulate

forms, respectively. While approximately 50% of the TP in freezing works effluent was present as DIP, the majority of the N was in dissolved organic (48%) and particulate (28.5%) forms. The majority of the P in dairy shed effluent was present in particulate forms and change in climatic conditions did not appreciably alter the ratios of P forms. The proportions of N forms, however, were appreciably altered. Whereas 40% of the TN in the effluent sampled in early summer was particulate N, only 18% of the TN was present in this form in the winter samplings. The proportion of N present as NO_3-N showed a four-fold decrease from the winter to early summer sampling runs (17% - 4%).

5. Correlation coefficients between A_{250nm} and various organic or organically-related parameters indicated that absorption at this wavelength could be developed as a predictor for use in effluent surveillance programmes. A_{413nm} gave superior correlation coefficients with dissolved inorganic constituents in freezing works effluent and deserves further investigation.

6. On the basis of methodological considerations, the use of the COD test rather than the BOD_5 test for routine monitoring of effluent strength is to be recommended.

7. Discussions of hydrographs, and concentration and loading graphs illustrated the point that effluent sampling is not straightforward. Detailed studies of effluent types need to be made in order to make recommendations for stratified systematic sampling. The two approaches demonstrated for the determination of sampling frequency are suitable for both characterisation studies, and the formulation of effluent compliance sampling programmes for Regional Water Boards.

APPENDIX 1 Survey on monitoring of effluent discharges

This questionnaire was sent to all Regional Water Boards in the country. Board officers were asked to answer the majority of questions by ticking the appropriate boxes provided. In the interests of economy, these boxes have been omitted and some of the questions rephrased slightly, in order to make sense in the present context.

1. List the ten major effluent discharge sources in your area. Include the name of the discharger, type of effluent, volume of permissible discharge per day, BOD loading permissible per day, and body of water into which effluent is discharged.
NOTE. The ten major effluent discharges may be assessed subjectively. For example, a particular discharge may have a low organic loading but may be considered major on the basis of toxic components.

2. Do you sample each of these effluents: (a) Routinely, (b) Only if you suspect the discharger is violating his right, (c) Not at all?

3. Indicate which of the following sampling techniques you employ when sampling effluent discharges: (a) Grab, (b) Flow-weighted composite, (c) Time-weighted composite, (d) Automatic discrete, (e) Automatic continuous, (f) Other (please specify). (If more than one used please rank in order of frequency.)

4. If automatic sampling was specified in question 3, what brand and type of sampler do you use? EXAMPLE ISCO, 28 discrete sample capacity, peristaltic pump mechanism.

5. In the routine monitoring of any particular effluent discharge, indicate which of the following would best describe the frequency with which a sampling exercise is undertaken: (a) Daily, (b) Weekly, (c) Monthly, (d) 3-monthly, (e) 6-monthly, (f) Yearly, (g) Cannot define but irregularly.

6. During any particular sampling exercise, which of the following would best describe the number of samples usually taken on a daily basis? (a) 1, (b) 2-3, (c) 4-6, (d) 7-12, (e) 13-24, (f) > 24.

7. Do you routinely sample effluent discharges outside of normal working hours?

8. With respect to the ten major discharges outlined in question 1, indicate whether you have made any assessment of the annual loading of the parameters specified in each water right.

9. Are flowmeters installed on any or all of the ten major discharges? If yes, please indicate whether recording or instantaneous flow meters are installed at each discharge.

10. Indicate whether any or all of the ten major dischargers report the results of any self-monitoring to the RWB.

11. Indicate which of the following chemical and microbiological analyses you carry out or have carried out on the effluents in question 1. If a particular analysis is carried out only on selected effluents, please write the letter corresponding to the effluent beside the parameter.

I	Biological oxygen demand
II	Chemical oxygen demand
III	Dissolved oxygen
IV	Total solids
V	Suspended solids
VI	pH
VII	Temperature
VIII	Total coliforms
IX	Faecal coliforms
X	Faecal streptococci
XI	Dissolved inorganic phosphorus
XII	Total phosphorus
XIII	Ammonium-nitrogen
XIV	Nitrate-nitrogen
XV	Total nitrogen (or Kjeldahl-nitrogen)
XVI	Heavy metals (Zn, Cd, Hg, etc.) please specify.
XVII	Other toxic substances (cyanides, phenols, etc.) specify.

12. Which of the chemical and microbiological parameters listed in question 11 does your Board have facilities to carry out and which are, or have been, contracted out?

13. Do any of the ten major effluents in question 1 have any nutrient

limitations (i.e., nitrogen or phosphorus forms) included in their discharge rights? If so, please state the limitation.

14. Do any of the ten major effluents listed have any limitations with regard to toxic substances (i.e., heavy metals, cyanides, arsenic, phenols, etc.) included in their discharge rights? If so, please state the limitation.

15. Which of the following would best represent the length of time between sampling and start of analysis for each of the parameters listed in question 11? (a) < 1 hr. (b) 1-3 hr. (c) 3-6 hr. (d) 6-12 hr. (e) 12-24 hr. (f) > 1 day.

16. For each of the parameters listed in question 11, which of the following methods of preservation are routinely used if any delay is incurred between sampling and analysis? (a) None, (b) Refrigeration, (c) Freezing, (d) Preservative addition.

17. If any preservative addition was specified in question 16, please name the preservative(s) used.

18. Outline any special circumstances or limitations which may be peculiar to your local situation with regard to the routine sampling and analysis of effluent samples.

19. Please feel free to add any further comments which you consider may be helpful to this survey.

APPENDIX 2-1

Effect of storage treatments and time of storage on the dissolved inorganic phosphorus concentration of effluent samples.

Effluent	Storage temperature	Amendment	Storage time (days) 0	2	4	8	10	17	22	30
			DIP ($mg.\ell^{-1}$)							
Municipal sewage effluent	Room	-	1.65	1.46	1.27	1.42	1.35	1.43	1.47	1.40
	Room	N-Serve		1.37	1.13	1.40	1.33	1.45	1.45	1.37
	Room	$NgCl_2$		1.56	1.49	1.61	1.54	1.62	1.58	1.56
	4 C	-		1.58	1.48	1.66	1.49	1.49	1.43	1.45
	4 C	N-Serve		1.42	1.28	1.39	1.35	1.43	1.37	1.38
	4 C	$HgCl_2$		1.53	1.43	1.65	1.57	1.60	1.56	1.56
	-10 C	-		1.55	1.54	1.58	1.56	1.70	1.78	1.71
	-10 C	N-Serve		1.50	1.39	1.51	1.64	1.62	1.52	1.50
	-10 C	$HgCl_2$		1.56	1.47	1.60	1.43	1.72	1.49	1.55
Freezing works effluent	Room	-	3.88	3.94	0.78	1.29	1.82	3.07	2.53	
	Room	N-Serve		4.00	0.82	2.06	1.80	3.16	3.01	
	Room	$HgCl_2$		4.24	4.25	2.88	3.69	3.07	3.30	
	4 C	-		5.01	3.61	3.11	2.44	2.55	2.40	
	4 C	N-Serve		4.75	3.78	3.63	2.32	2.46	2.80	
	4 C	$HgCl_2$		4.28	3.90	4.35	4.68	4.38	4.04	
	-10 C	-		3.82	3.70	4.00	4.04	3.56	3.77	
	-10 C	N-Serve		4.31	4.02	4.66	4.38	4.44	4.02	
	-10 C	$HgCl_2$		3.94	3.39	4.02	4.07	4.08	4.02	

APPENDIX 2-2

Effect of storage treatments and time of storage on the dissolved ammonium nitrogen concentration of effluent samples.

Effluent	Storage temperature	Amendment	Storage time (days)							
			0	3	5	9	12	16	22	29
			DNH_4-N ($mg.\ell^{-1}$)							
Municipal sewage effluent	Room	-	14.9	14.8	14.8	13.4	15.3	11.0	6.5	0.5
	Room	N-Serve		13.9	13.7	15.6	13.7	14.5	13.9	11.3
	Room	$HgCl_2$		14.2	14.5	13.2	13.0	13.8	13.8	13.6
	4 C	-		16.2	16.3	14.1	14.6	15.3	14.1	16.2
	4 C	N-Serve		13.6	13.7	14.0	13.1	14.3	13.3	14.0
	4 C	$HgCl_2$		14.1	13.3	14.3	13.5	13.8	13.2	12.7
	-10 C	-		14.7	14.6	14.3	14.6	13.9	13.8	
	-10 C	N-Serve		11.6	11.8	13.4	13.4	8.1	12.4	
	-10 C	$HgCl_2$		14.5	9.7	13.8	13.9	9.3	11.8	
Freezing works effluent	Room	-	46.3	61.7	35.1	45.2	51.8	49.5	52.6	52.2
	Room	N-Serve		43.3	36.4	46.0	51.2	51.3	53.4	54.9
	Room	$HgCl_2$		50.0	47.5	46.5	44.9	36.8	38.2	40.5
	4 C	-		48.4	44.0	43.8	42.6	39.5	42.4	40.0
	4 C	N-Serve		50.7	50.3	44.8	41.6	45.8	46.1	40.0
	4 C	$HgCl_2$		49.5	48.9	49.7	50.8	47.7	48.0	47.0
	-10 C	-		38.2	39.6	46.3	39.6	41.6	42.2	43.8
	-10 C	N-Serve		46.2	35.6	46.1	33.8	37.1	35.4	36.0
	-10 C	$HgCl_2$		55.6	44.3	47.9	48.5	46.8	49.6	50.0

APPENDIX 2-3

Effect of storage treatments and time of storage on the total ammonium nitrogen concentration of effluent samples.

Effluent	Storage temperature	Amendment	Storage time (days) 0	3	5	9	12	16	22	29
			TNH_4-N ($mg.\ell^{-1}$)							
Municipal	Room	-	15.0		15.3	12.4	14.1	10.2	2.3	0.5
sewage	Room	N-Serve		14.9	15.2	13.0	17.7	17.5	15.3	14.6
effluent	Room	$HgCl_2$		14.6	14.5	13.3	13.7	14.1	12.7	13.0
	4 C	-		15.3	14.1	13.7	14.2	14.7	14.3	16.2
	4 C	N-Serve		14.0	13.4	13.1	12.5	15.3	13.6	16.2
	4 C	$HgCl_2$		14.9	14.4	13.7	13.5	13.2	13.6	13.4
	-10 C	-		14.8	13.4	14.4	12.7	13.2	12.9	
	-10 C	N-Serve		12.5	12.5	12.2	12.7	12.4	11.1	
	-10 C	$HgCl_2$		14.3	13.4	14.3	13.8	13.2	11.8	
Freezing	Room	-	50.9	63.7	53.1	51.2	63.9	61.2	53.1	52.1
works	Room	N-Serve		51.2	48.2	59.2	62.5	63.5	63.1	58.5
effluent	Room	$HgCl_2$		52.5	49.9	58.5	58.2	62.8	58.2	57.4
	4 C	-		53.8	51.7	52.5	47.5	44.3	54.2	58.5
	4 C	N-Serve		55.8	53.4	55.7	44.2	45.8	46.9	47.7
	4 C	$HgCl_2$		55.4	53.3	52.2	49.3	48.9	55.7	52.1
	-10 C	-		52.2	43.8	45.2	45.8	44.9	44.2	44.0
	-10 C	N-Serve		55.5	34.2	50.8	49.5	46.6	44.5	43.6
	-10 C	$HgCl_2$		55.6	44.3	47.9	48.5	46.8	49.6	50.0

APPENDIX 2-4

Effect of storage treatments and time of storage on the dissolved nitrate nitrogen concentration of effluent samples.

Effluent	Storage temperature	Amendment	Storage time (days)							
			0	3	5	9	12	16	19	29
			DNO_3-N ($mg.\ell^{-1}$)							
Municipal sewage effluent	Room	-	0.16	0.09	0.13	0.19	0.13	3.43	6.16	8.30
	Room	N-Serve		0.12	0.14	0.20	0.13	0.19	0.28	1.20
	Room	$HgCl_2$		0.13	0.10	0.08	0.27	0.20	0.10	0.16
	4 C	-		0.06	0.10	0.17	0.01	0.08	0.05	0.13
	4 C	N-Serve		0.08	0.13	0.12	0.14	0.21	0.23	0.20
	4 C	$HgCl_2$		0.05	0.10	0.10	0.07	0.10	0.30	0.17
	-10 C	-		0.07	0.07	0.08	0.07	0.10	0.06	0.07
	-10 C	N-Serve		0.07	0.07	0.08	0.08	0.08	0.10	0.10
	-10 C	$HgCl_2$		0.07	0.08	0.07	0.04	0.05	0.07	0.07
Freezing works effluent	Room	-	0.14	0.02	0.02		0.02	0.07	0.03	0.02
	Room	N-Serve		0.03	0.02		0.02	0.10	0.08	0.08
	Room	$HgCl_2$		0.11	0.13		0.12	0.16	0.16	0.13
	4 C	-		0.05	0.14		0.02	0.06	0.03	0.06
	4 C	N-Serve		0.07	0.09		0.07	0.05	0.03	0.03
	4 C	$HgCl_2$		0.04	0.09		0.08	0.10	0.09	0.09
	-10 C	-		0.05	0.08		0.08	0.06	0.08	0.08
	-10 C	N-Serve		0.12	0.06		0.07	0.05	0.05	0.06
	-10 C	$HgCl_2$		0.05	0.06		0.06	0.05	0.05	0.05

APPENDIX 2-5

Effect of storage treatments and time of storage on the dissolved Kjeldahl-nitrogen concentration of effluent samples.

Effluent	Storage temperature	Amendment	Storage time (days)							
			0	2	4	8	10	18	23	30
			DKN ($mg.\ell^{-1}$)							
Municipal Sewage effluent	Room	-	16.3	16.6	15.6	16.5	17.8	9.1	7.4	2.7
	Room	N-Serve		20.0	23.7	17.0	16.0	18.3	18.1	15.8
	Room	$HgCl_2$		15.4	16.3	16.0	15.7	17.7	18.4	15.0
	4 C	-		16.0	17.0	16.0	17.0	15.7	16.4	15.8
	4 C	N-Serve		15.1	15.8	15.5	17.1	14.3	16.6	15.5
	4 C	$HgCl_2$		16.2	16.8	15.5	17.0	15.4	16.4	16.5
	-10 C	-		14.3	15.8	14.0	16.4	14.5	14.8	14.7
	-10 C	N-Serve		11.4	11.4	10.9	17.7	13.1	12.7	14.0
	-10 C	$HgCl_2$		12.8	12.3	11.4	11.6	15.1	15.7	9.5
Freezing works effluent	Room	-	65.3	55.4	66.1	68.2	64.3		67.6	62.0
	Room	N-Serve		60.3	67.6	69.3	60.2	59.4	65.7	51.7
	Room	$HgCl_2$		63.7	70.9	55.3	67.8	56.0	70.6	59.3
	4 C	-		60.8	69.4	59.5	68.4	65.1	58.6	62.1
	4 C	N-Serve		68.0	68.5	62.7	69.8	61.7	58.6	63.3
	4 C	$HgCl_2$		63.1	72.6	61.8	71.2	60.8	64.8	66.5
	-10 C	-		46.9	65.2	55.9	65.7	46.3	60.0	58.1
	-10 C	N-Serve		49.4	65.2	54.6	58.9	58.3	42.9	56.3
	-10 C	$HgCl_2$		56.8	52.2	58.1	49.2	48.6	58.5	61.5

APPENDIX 2-6

Effect of storage treatments and time of storage on the total Kjeldahl-nitrogen concentration of effluent samples.

Effluent	Storage temperature	Amendment	Storage time (days)							
			0	2	4	8	10	18	23	30
			TKN ($mg.\ell^{-1}$)							
Municipal sewage effluent	Room	-	18.0	19.7	17.8	18.3	19.8	3.6	3.4	3.5
	Room	N-Serve		17.4	17.5	19.3	18.8	19.1	17.2	17.2
	Room	$HgCl_2$		21.4	19.0	19.1	20.1	19.7	19.1	18.7
	4 C	-		18.5	19.0	17.2	19.1	16.0	17.1	17.0
	4 C	N-Serve		18.3	17.3	16.8	19.8	17.7	17.7	18.2
	4 C	$HgCl_2$		20.0	21.0	16.8	20.1	18.6	17.7	17.0
	-10 C	-		16.0	16.8	15.0	19.8	16.8	17.6	18.8
	-10 C	N-Serve		13.4	15.8	14.8	21.2	18.6	21.1	16.7
	-10 C	$HgCl_2$		15.1	16.8	15.7	18.4	17.4	14.9	16.0
Freezing works effluent	Room	-	90.9	69.1	60.7	65.0	82.8	76.6	80.0	58.1
	Room	N-Serve		74.8	70.1	66.0	80.1	80.0	80.5	76.1
	Room	$HgCl_2$		73.2	67.2	70.2	77.3	88.6	74.2	76.7
	4 C	-		77.7	73.1	78.5	86.3	79.3	75.6	83.6
	4 C	N-Serve		77.7	74.1	80.9	83.5	88.0	88.9	86.6
	4 C	$HgCl_2$		73.7	81.5	89.1	78.0	84.5	83.4	83.7
	-10 C	-		62.9	66.7	78.0	79.4	71.4	72.2	75.5
	-10 C	N-Serve		62.8	74.1	73.6	85.6	82.9	63.3	62.2
	-10 C	$HgCl_2$		64.0	68.6	80.0	80.8	70.8	53.9	57.5

APPENDIX 3 Growth characteristics of bacterial species in effluent samples stored at room temperature and 4 C for 24 hours.

		Total coliforms ($n.m\ell^{-1} \times 10^5$)		Faecal coliforms ($n.m\ell^{-1} \times 10^3$)		Faecal streptococci ($n.m\ell^{-1} \times 10^2$)	
	Time (hours)	Room	4 C	Room	4 C	Room	4 C
A Sewage effluent	0	2.6	2.6	7.3	4.7	35	35
	3	7.3	2.6	2.6	6.3	36	36
	6	12	2.1	15	8.6	36	33
	9	18	1.5	15	4.2	28	27
	12	16	1.7	14	2.0	34	28
	24	13	3.0	17	8.0	26	29
B Dairy shed effluent	0	6.9	5.8	8.8	8.6	4.2	4.2
	3	8.8	4.0	9.5	9.3	4.1	3.0
	6	10	3.8	1.4	8.0	30	4.6
	9	18	4.5	15	10	47	4.5
	12	24	3.0	21	2.3	87	8.5
	24	31	12	11	9.5	64	4.6
C Freezing works effluent	0	35	38	64	63	200	180
	3	44	29	61	60	150	260
	6	54	31	59	52	180	200
	9	59	33	48	38	160	170
	12	65	38	40	33	100	190
	24	42	38	62	27	110	280

Appendix 4 Rainfall recorded at Palmerston North (Massey University) on effluent sampling days.*

Effluent sampled	Sampling run	Date	Rainfall (mm)
Municipal sewage	S1	23/6/76	0.0
		24/6/76	1.0
	S2	9/8/76	0.3
		10/8/76	4.0
	S3	9/12/76	7.5
		10/12/76	0.0
Dairy shed	D1	3-day antecedent	47.3
		16/9/76	1.5
		17/9/76	2.7
	D2	3-day antecedent	0.0
		24/11/76	9.1
		25/11/76	0.0
	D3	3-day antecedent	0.0
		29/11/76	1.4
		30/11/76	0.0

*Rainfall data are not relevant for freezing works effluent sampling runs as effluent flow is unaffected by rainfall.

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