Wastewater Disinfection Field Trials

WRc plc

R&D Project Report 231/4/SW

WASTEWATER DISINFECTION FIELD TRIALS

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SUMMARY

This report describes work investigating the efficacy and environmental impact of some sewage disinfection methods in full scale field trials. Water Utilities are considering disinfection of screened sewage outfalls or secondary effluent discharges from coastal or inland sewage treatment works, as the NRA take steps to enable bathing waters to meet EC bathing water directive standards and protect bathers from pathogenic micro-organisms.

Three aspects of sewage disinfection were considered: by-product formation of disinfection, the ability of the disinfectants to reduce the numbers of both indicator and potentially pathogenic micro-organisms, and the ecotoxicity of sewage before and after it had been disinfected. There were three full scale field trials in 1990. The first looked at the efficacy and effects of ultraviolet radiation on a secondary effluent from a sewage treatment works. The other two studied the effects and impact of peracetic acid, on a fine screened raw sewage discharge and a biologically treated effluent respectively.

Overall, ultraviolet light looks a promising method for disinfecting secondary effluent, though there is a need for more work in the field on the potential for photoreactivation of indicator micro-organisms and on the disinfection efficacy for target potential pathogens. The work on peracetic acid did not suggest that it performed well as a disinfectant of fine screened sewage, though it seemed to be generally effective against indicator micro-organisms in effluent that had previously received full secondary biological treatment. The secondary effluent work cast doubt on the ability to accurately monitor and maintain peracetic acid at required concentrations.

All three trials demonstrated the need to develop non-routine microbiological analysis techniques for potentially pathogenic bacteria and viruses as they relate to sewage, effluent and environmental waters.

KEY WORDS

Sewage disinfection, peracetic acid, ultraviolet radiation, PAA, UV, ecotoxicology.

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1. INTRODUCTION

1.1 Background

Conventional wastewater treatment for domestic sewage has been directed towards alleviating aesthetic problems and at reducing the oxygen demand imposed by discharges. Effluent from traditional sewage treatment works (STW) contains high numbers of micro-organisms and, at present, relocation of sewage discharges to more remote positions is the only available means of protecting the microbial quality of bathing, recreational or shellfish rearing waters. This often requires a long sea outfall, which is sometimes impractical or inappropriate. If, for example, local topography or large numbers of seasonal tourists have a significant effect, then disinfection may be the only viable solution to existing waste disposal problems.

The EC Bathing Water Directive (CEC, 1976) controls the numbers of micro-organisms permitted in bathing waters. This places the limits on five micro-organisms (Table 1.1). However, while there is no direct legislation covering the numbers of indicator organisms in a sewage works, the NRA (as the discharge consenting authority in England and Wales) can place controls on the numbers of these organisms present to ensure that an adjacent bathing and/or shellfish water meets the standards set by the Directives. This includes those situations where a sewage works discharges into a river which then discharges into a bathing or shellfish rearing water.

Table 1.1 - Limits on five micro-organisms set in the EC Bathing Waters Directive

	Guide value	Mandatory
Total coliforms per 100 ml	500 (80)	10,000 (95)
Faecal coliforms per 100 ml	100 (80)	2,000 (95)
Faecal streptococci per 100 ml	100 (90)	-
Salmonella per 1 litre	-	0 (95)
Enteroviruses per 10 litres	-	0 (95)

The figures in brackets indicate percentage compliance

The EC Shellfish Waters Directive (CEC 1979) limits the numbers of faecal coliforms permitted in the shellfish flesh and intervalvular fluid to 300 per 100 ml. An EC Directive on Shellfish Hygiene was agreed by the Council of Ministers on 16 July 1991, which requires all Member States to classify their shellfish harvesting areas into one of three categories according to the degree of faecal indicator bacteria present in samples of shellfish flesh. The Directive sets threshold limits for faecal coliforms in shellfish and the appropriate treatment regimes for contaminated shellfish (Table 1.2). The mandatory limit for shellfish intended for direct human consumption is 230 *E. coli* or 200 faecal coliforms per 100 g of shellfish flesh.

Table 1.2 - Limits on faecal coliforms in EC Directive on Shellfish Hygiene

Threshold limit of faecal coliforms per 100 g of shellfish flesh	Levels of treatment required prior to consumption
<300	May go for direct human consumption
300 - 6000	Must be depurated, heat treated or relaid
6000 - 60 000	Must be relaid for a long period (at least 2 months)
>60,000 or a lesser figure at the discretion of the Member State	Prohibited

Against this regulatory background the water utilities announced in 1989 a £1.4 billion investment programme to improve the quality of EC bathing waters. The recent adoption of the EC Directive on Urban Wastewater Treatment (CEC 1991), which prescribes the minimum level of treatment discharges should receive, is likely to add a further £1.5 billion to the investment required.

There is a pressing need to find disinfection techniques which will satisfy EC obligations and give the best return on the massive investments, but which are also environmentally acceptable. Bathing water improvements must be achieved

by the latter half of the 1990s and the scale of the engineering task ahead imposes a degree of urgency on the identification of acceptable processes.

1.2 Objectives

This project investigated the efficacy and environmental impact of some disinfection methods in full scale field trials. It links closely with the laboratory studies of NRA project 38 (Crathorne *et al* 1991), which looked at sewage disinfection by-product formation, ecotoxicology and microbiological efficacy.

The overall objective of these 3 trials was the full scale evaluation of disinfection methods for fine screened sewage and secondary effluent flows. The trials were a collaborative project funded by South West Water Services Ltd, the NRA and Water Service plc's as part of WRc's Common Interest Research Programme (CIRP); they were coordinated by a Disinfection Trials Working Group, which was set up in 1989. The group has representatives from WRc plc, South West Water Services Limited, the National Rivers Authority and the Ministry of Agriculture, Fisheries and Food. These trials form part of a wider programme of collaborative trials whose detailed objectives are to evaluate:

(i) the performance of a range of disinfectants for a number of situations to include crude sewage, stormwater and secondary effluent discharges to river, estuary or marine locations;

(ii) the optimisation of process performance and process control;

(iii) capital and operating costs of different processes;

(iv) the suitability of individual disinfection techniques for use with sewage, stormwater, primary and secondary effluent treatment;

(v) the effect of disinfection on the receiving water environment.

The NRA's specific objectives were:

(i) to determine the effect on organisms for which standards have been laid down by the EC Bathing Water Directive;

(ii) to determine the effect on target potentially pathogenic organisms;

(iii) to investigate the reversibility of (apparent) mortalities amongst these organisms;

(iv) to identify the degradation products and waste by-products of the processes;

(v) to assess the ecotoxicological impact of the disinfected sewage.

However, discharge consents set by the NRA are increasingly taking account of the performance-specific processes involved, so the NRA also had an interest in the investigations carried out on process-related factors associated with disinfection.

1.3 Disinfection processes and sites studied

There are a number of sewage disinfection processes that the water industry are considering. There are four main approaches to disinfection:

(i) **Oxidation methods** Oxidising agents such as chlorine, chlorine dioxide, sodium hypochlorite, ozone and peracetic acid;

(ii) **Filtration** Disinfection may be achieved by micro-filtration processes which effectively apply tertiary treatment;

(iii) **Denaturation** The denaturation of organic matter, including the proteins and nucleic acids of bacteria and viruses, can be accomplished by ultraviolet (UV) irradiation;

(iv) **Enhanced primary treatment** (e.g. the Clariflow process).

Comprehensive reviews of available methods of disinfection have been published (CES 1988; Thomas and Dillon 1989). The studies reported here focus on two of the methods: treatment with peracetic acid (PAA) and ultraviolet radiation (UV). This report discusses the findings of three studies, that are described in detail in their own interim reports.

There were two studies on the effects of PAA. The first was on fine screened sewage (1.5 mm) discharging to sea at Trevaunance Cove, St Agnes (Roddie *et al* 1991a). The other studied a biologically treated secondary effluent discharging to a freshwater stream above Porthtowan Beach (Roddie *et al* 1991b). The study of the effect of UV took place at Menagwins STW, St Austell, where a secondary treated effluent discharges to a freshwater course 4 km upstream of Pentewan Sands Beach (Realey and Brogden, 1991).

Before the reviews of each of the trials, this report provides a discussion of the disinfection techniques used and the analytical determinands measured during the trials.

2. MODES OF ACTION OF DISINFECTANTS

2.1 Ultraviolet radiation

Thomas and Dillon (1989) discussed the action of UV radiation on wastewater in their review of wastewater disinfection. Inactivation of organisms by UV radiation results primarily from the absorption of radiation by the nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), contained in the microbial cells. Nucleic acids demonstrate maximum absorption when exposed to UV radiation between 250-265 nm. The energy associated with UV radiation induces photochemical changes in the nucleic acids, particularly the dimerisation of thymine bases in the DNA. Formation of thymine dimers distorts the double helix structure of DNA and may prevent replication, which results in the death of the cell. Alternatively, replication may proceed, producing a mutant daughter cell, which itself is unable to replicate.

Exposure of UV damaged cells to light in the visible region, may repair some of the damage to the DNA. This phenomenon has been termed photoreactivation and occurs primarily in the wavelength dependent on the particular organism. Reactivation of UV damaged cells can also occur in the dark. Micro-organisms shown to repair in this way include Streptomyces, *Eschericia coli*, Saccharomyces, Aerobacter, Micrococcus, Erwinia, Proteus, Penicillium, and Neurospora. Viruses generally do not photoreactivate, except when in a host cell which can repair. Some suppliers of UV equipment allow for an order of magnitude rise in bacterial numbers due to photoreactivation, in their design of wastewater disinfection systems.

2.2 Peracetic acid

Peracetic acid (PAA), $CH_3CO.OOH$, is manufactured and supplied in the UK by Interox Chemicals Limited of Warrington under the trade name of 'Oxymaster'. Oxymaster is an equilibrium mixture of peracetic acid, water, hydrogen peroxide and acetic acid:

$$CH_3CO.OH + H_2O_2 = CH_3CO.OOH + H_2O$$

PAA and hydrogen peroxide decompose as follows:

$$CH_3CO.OOH \rightarrow CH_3CO.OH + \tfrac{1}{2} O_2$$

$$H_2O_2 \rightarrow H_2O + \tfrac{1}{2} O_2$$

Oxymaster has a shelf life of approximately one year, and the dose is usually described in mg l^{-1} of PAA.

One of the main advantages of using PAA is that the process requirements are minimal. Only chemical storage, pumping facilities and some suitable control apparatus are necessary. Manual or flow-proportional control is usually sufficient to control PAA dosage, though there is a fully automated residual control system for large installations. Dosing comprises in-line injection either through a single point or multiple injection points, such as a sparge. The receiving effluent must then be thoroughly mixed, though the natural turbulence of the receiving liquor often suffices.

Interox claim that the inactivation of indicator organisms such as thermotolerant coliforms is rapid (less than ten minutes).

3. SAMPLING AND ANALYSIS OF CHEMICAL AND MICROBIOLOGICAL DETERMINANDS

This chapter reviews the chemical and microbiological parameters studied in each of the three field trials. The ecological and ecotoxicological studies performed differed for each of the trials, so they are described in the relevant chapter. Those chapters also describe the sampling regimes and dosing rates followed for the particular disinfectant and substrate.

3.1 Routine microbiology

The routine microbiological parameters measured were numbers of thermotolerant coliforms (TTC) (more commonly known as faecal coliforms) and faecal streptococci (FS). These are the indicator micro-organisms for which there are limits set in the EC Bathing Waters Directive (see Section 1.1). There is some concern that they do not adequately reflect the behaviour of other microbiological pathogens that may be present, so the field trials also comprised measurements of the non-routine microbiological parameters described in Section 3.3.

Routine microbiological analyses were carried out on-site. All samples were analysed for TTC. Aliquots of samples diluted on 0.1% peptone broth were filtered through cellulose membrane filters (average pore diameter 0.45 µm). Filters were placed in lauryl sulphate broth and incubated at 30 °C for 4 hours, followed by 14 hours at 44 °C. Coliform bacterial numbers were estimated from the number of yellow colonies present on the filters after incubation.

Faecal streptococcal numbers were estimated in a similar way, from the number of red colonies present on filters incubated on Slantetz and Bartley medium (4 hours at 37 °C followed by 44 hours at 44 °C).

3.2 Chemistry

Table 3.1 lists the wide range of organic, inorganic and physical determinations for which samples were taken. Most analyses were carried out using standard

water industry techniques. Those that were non-routine are described in the following sections that relate to that determinand.

Table 3.1 - Chemical determinands

Determinand
BOD
COD
pH
Phosphate
Total oxidisable N
NH_3-N
Suspended solids
Ca
K
Na
Mn
Mg
B
Fe
Al
Si
SiO_2
S
SO_2
Cu
Zn
Pb
Cr
Ni
Cd
Volatile organohalogen (VOX)
Residual oxidants (RO)
Complex organics (GCMS)
Phenol and chlorinated phenols (P/CP)
Adsorbable organohalogen (AOX)

3.2.1 Volatile organohalogens

Samples were collected in 20-ml vials fitted with septum caps. These were filled to capacity, ensuring that no air was included. The analytical method described below is suitable for the analysis of raw and potable waters. Fifteen ml of sample was extracted with 10 ml of pentane. The volatile halogenated solvents (VOXs) in the extract were separated by gas chromatography and detected with an electron capture detector. Concentrations were calculated using an internal standard, by comparing the individual VOX peaks areas with the peak areas for known quantities of standard.

The method determines:

		*
$CHCl_3$	Chloroform	0-90 µg l^{-1}
$CHCl_2Br$	Bromodichloromethane	0-60
$CHClBr_2$	Dibromochlormethane	0-92
$CHBr_3$	Bromoform	0-87
111-$C_2H_3Cl_3$	1,1,1-trichloroethane	0-90
CCl_4	Carbon tetrachloride	0-30
C_2HCl_3	Trichloroethylene	0-90
112-$C_2H_3Cl_3$	1,1,2-trichloroethane	0-90
C_2Cl_4	Tetrachloroethylene	0-30
C_6H_5Cl	Chlorobenzene	0-4.5
12-$C_6H_4Cl_2$	1,2-dichlorobenzene	0-300
13-$C_6H_4Cl_2$	1,3-dichlorobenzene	0-300
14-$C_6H_4Cl_2$	1,4-dichlorobenzene	0-300
12-$C_2H_4Cl_2$	1,2-dichloroethane	0-300

* Range of application

3.2.2 Phenol and chlorinated phenols

Samples were collected in 250 ml glass vessels to which 0.5 ml concentrated H_2SO_4 had been added as a preservative. Samples were made alkaline and pre-extracted with hexane to reduce the levels of interfering compounds. Each sample was buffered using sodium bicarbonate and shaken with a solution of pentaflurobenzoylchloride in hexane. The phenol pentaflurobenzoyl esters partition into the hexane, and excess reagent was removed by rinsing with NaOH solution. The extracts were separated using capillary gas chromatography (CGC)

and detected by electron capture detector. Phenol concentrations were calculated using a chromatography data system either by means of external standards or by manual measurement of peak height.

3.2.3 Adsorbable organohalogens (AOX)

Samples were collected in 100 ml glass bottles, filled to capacity. No preservative was necessary.

3.2.4 Complex organics (GCMS)

To commence the solvent extraction of organic contaminants from the batches of sewage for GCMS analysis, 100 ml of dichloromethane was added to each bottle immediately after sampling, at the sampling site. In the laboratory, each sample was spiked with deuterium labelled standard compounds to give concentrations of 1 μg l^{-1} and two extractions carried out using DCM, one under basic conditions and one under acidic conditions.

Initially, the samples were made basic to pH 10 by the addition of sodium hydroxide solution and extracted by shaking with the DCM that was originally added at the time of sampling. Thick emulsions were formed which were separated by centrifugation. The DCM layer was collected and then the aqueous phase re-extracted with a further 100 ml aliquot of DCM.

The samples were then made acidic to pH 2 by the addition of sulphuric acid. The extraction of the samples was repeated with 2 x 100 ml portions of DCM. Thick emulsions were also obtained during the acidic extractions and centrifugation was required in order to obtain a separation of organic and aqueous phases. The acidic and basic extracts were stored in a freezer. Water present in the extracts was frozen out and the ice filtered off. The extracts were concentrated to 1 ml using Kuderna-Danish and nitrogen blow-down apparatus. The acidic fraction of each sample was methylated using freshly generated diazomethane and then 10 μg l^{-1} of d_{10}-atrazine was added to each extract to provide additional internal standardisation. One QA/QC laboratory

blank extract was prepared from clean borehole water for each batch of samples using the same procedures that were used for the samples.

GC-MS analysis was carried out using a Hewlett Packard 5890 GC equipped with a 60 m x 0.32 mm DB-1 fused silica capillary column and cool on-column injector. The GC was connected to a VG 7070E double focusing magnetic sector MS via a heated direct interface. The MS was equipped with an electron impact (EI) source which was operated in the full scan positive EI mode at a temperature of 250 °C.

GC-MS analysis was achieved using the following operating conditions:

GC temperature programme: 30 °C for 4 minutes, then 8 °C min^{-1} to 300 °C. Held for 20 minutes.

MS mass range: 700-20 d was scanned at a scan speed of 0.5 sec/decade giving a scan cycle time of 0.97 seconds. Electron energy was 70 eV, trap current was 100 µA and resolution was 1500. Mass calibration was achieved using perfluorokerosene.

Quantification was carried out by referring to the deuterated internal standards which were added prior to extraction; these were separated into the two fractions as shown below:

Basic fraction	Acidic fraction
d_6-benzene	d_{11}-hexanoic acid
d_8-toluene	d5-phenol
d_8-styrene	
d_3-1,1,1-trichloroethene	
d_5-chlorobenzene	
d_{10}-xylene	
d_8-naphthalene	
d_{34}-hexadecane	
d_{10}-phenanthrene	

On methylation, d_{11}-hexanoic acid was converted to the methyl ester and therefore this compound was selected as the internal standard for quantification of the acidic fraction.

3.2.5 Inorganics

Samples were collected in 100 ml polythene bottles containing 2 ml of 5 m nitric acid as a preservative. Either 100 ml of total sample or 100 ml of 0.45 µm filtrate was added. Where practicable, samples were filtered to provide an indication of the distribution of trace heavy metals between the dissolved and particulate phases. The filter units were rinsed with 10% nitric acid and deionised water after each sample.

Blank samples were processed to provide an indication of the contribution of the preservative and the filters to the measured values.

Samples were returned to the laboratory,and both total and filtered fractions were digested as follows:

Sample of 50 ± 5 ml and 5 ml HNO_3 were poured into an acid-cleaned beaker which was then covered with a watch glass. The solution was boiled down and then made up to 50 ml after quantitative transfer to a falcon tube.

Saline samples were not pre-treated prior to extraction and analysis.

Analysis of Ca, Mg, Na, K, Fe, Mn, Al, Cu, and Zn in sewage samples was carried out by Inductively Coupled Gas Plasma Atomic Emission Spectrometry (ICPAES). Cd, Cr, Ni and Pb in sewage samples were determined by Graphite Furnace Atomic Absorption Spectrometry (GFAAS).

In saline samples, Zn was determined by Flame Atomic Absorption Spectrometry (FAAS), Cr by GFAAS and Cu, Ni, Cd and Pb by GFAAS following an additional extraction procedure using ammonium pyrrolidine dithiocarbamate-III-trichloroethane, to facilitate detection of trace amounts.

Sewage samples collected at the same time as saline discharge plume samples (i.e. two samples on the second day of each sampling run) were also analysed by GFAAS and ICPAES using the same low-level technique as for the saline samples. Consequently the detection limits for these samples are considerably lower than for samples analysed by ICPAES using the standard technique without additional extraction.

Hardness in sewage samples was calculated from the Ca, Mg, and Si values and expressed as mg equivalent $CaCO_3$ per litre. SiO_2 was calculated from the Si value and SO_4 from the S value.

Limits of detection for each technique for trace heavy metals are:

Low-level ICPAES	Pb, Cu, Ni	0.1 µg l^{-1}
	Cd	0.01 µg l^{-1}
GFAAS	Pb, Ni	0.5 µg l^{-1}
	Cr, Cd	0.1 µg l^{-1}
ICPAES	Pb	50 µg l^{-1}
	Cd, Cu, Zn	4 µg l^{-1}
	Cr, Ni	10 µg l^{-1}

3.2.6 Residual oxidants

These were studied on the PAA trials at Porthtowan and Trevaunance Cove, but not the UV trial at Menagwins. The method provided by Interox for the measurement of residual PAA in sewage is based on the oxidation of chloride by PAA to hypochlorous acid and subsequent oxidation of DPD and absorbance measurement. This does not work very well, since PAA does not oxidise chloride, or only does so very slowly. The inability of PAA to oxidise chloride to chlorine was confirmed in laboratory experiments carried out as part of NRA project 38 (Crathorne *et al* 1991), which showed no production of organohalogen compounds when humic acid solutions containing chloride were treated with PAA, whereas PAA treatment of humic acid solutions with bromide resulted in the production of organohalogen compounds, indicating that PAA oxidised bromide to bromine. This finding provided the basis for the development of the PAA analytical technique, based on the DPD photometric technique (HMSO 1980).

Unlike chlorine, PAA did not react with DPD directly. However, it was found to react rapidly with potassium bromide (KBr) and potassium iodide (KI) to form bromine or iodine respectively, which in turn oxidised the DPD. Monochloramine (MCA) also reacted rapidly with KBr to oxidise DPD, indicating that it was unlikely that PAA could be measured separately from MCA. The possibility of adapting a method of chlorine dioxide analysis, based on the oxidation (decolouration) of Alizarin dye (Masschelein *et al* 1989), for the analysis of PAA without interference by MCA was evaluated. This method, however, was unsuitable; PAA did not appear to be reactive towards either Alizarin Violet 3R (Acid Chrome Violet K) or Alizarin Violet N under the conditions used.

Since a simple method which could be used in the field was required, the DPD/KI photometric technique was adopted for the analysis of PAA, although it was not possible to distinguish PAA from MCA. A calibration graph was prepared for PAA solutions ranging from 0.1 mg l^{-1} to 1 mg l^{-1} using DPD and KI and measuring absorbance at 604 nm. Where necessary, sewage samples were diluted to fall into this range. Free halogen (chlorine, bromine) and bromamines, if present, would interfere in the measurement of PAA, but this can be overcome either by adjusting the photometer to zero prior to addition of KI, or by taking a separate reading of uv absorbance prior to addition of KI. This latter reading will provide a measurement of free halogen including bromamines (White 1986). Whereas MCA cannot be differentiated from PAA using this technique, dichloramine (DCA) can be measured separately due to its slower reaction with KI.

There are problems with the measurement of disinfectant residuals in sewage, using a DPD titrimetric or photometric technique, because these techniques rely on detecting colour changes which may be obscured by the colour and turbidity of the sewage. The DPD/ferrous ammonium sulphate titrimetric method was unsuitable for sewage because visual recognition of the end point is required. However, it was possible to apply the DPD photometric technique after removal of particulate matter by centrifugation and by adjusting the absorbance reading of the sewage to zero prior to the addition of DPD. The need to centrifuge the sample causes a delay in analysing the sample, during which time disinfectants may carry on reacting; consequently the measured residuals may be lower than at the time of sampling. The latter is likely to be significant only if the

sample is taken after a short contact time, when disinfectant residuals are relatively high and reactions are still proceeding at a relatively fast rate.

Hydrogen peroxide (H_2O_2) analyses were carried out according to a published method (Bader *et al* 1988) which uses the peroxidase catalysed oxidation of DPD. A calibration graph was prepared for H_2O_2 concentrations ranging from 0.05 mg l^{-1} to 1.0 mg l^{-1}. The linear range was below about 0.3 mg l^{-1}; samples were diluted to fall into this range. To measure low concentrations of H_2O_2 in undiluted samples, it was necessary to centrifuge the sewage prior to analysis. The limit of detection depended on the dilution of the sample; it was about 0.01 mg l^{-1} for undiluted, centrifuged sewage and correspondingly higher for diluted sewage. An experiment was carried out to establish that PAA did not interfere in the H_2O_2 measurement.

3.3 Non-routine microbiology

3.3.1 Review of microorganisms studied

In addition to the routine microbiological determinands (thermotolerant coliforms and faecal streptococci) 7 other groups of more robust organisms were studied during the course of the trial. These were:

Enterovirus;
Rotavirus;
Salmonella;
Pseudomonas aeuriginosa;
Staphylococcus aureus;
Campylobacter;
F^+ Coliphage.

Enterovirus and Salmonella are included in the EC Bathing Water Directive. One of the chief concerns over disinfection is that it kills only the indicator organisms and not the pathogens present in sewage. The main purpose of carrying out this work was to get some idea of the effects of the disinfectants on a wider range of organisms. The rest of this section describes the

different micro-organisms and the reasons for measuring their levels in disinfected sewage. Chapter 7 discusses the performance of these studies for all three trials and the suitability of available test methods.

(a) Enterovirus

Enterovirus are part of the picornavirus group and include polioviruses, coxsackieviruses and echoviruses. They are all found in the intestines and are excreted in faeces. Infections associated with enteroviruses include polio, meningitis and hepatitis A.

Enterovirus are small (hence the name picorna) e.g. a polio virus is approximately 27 nm in diameter. Sixty-eight serotypes of enterovirus have been identified in the water cycle.

(b) Rotavirus

Rotavirus are part of the reovirus family. They are believed to be associated with up to 80% of all cases of viral gastroenteritis and are a major cause of death in infants and young children. Three serotypes effect man, these are groups A, B and C. Group A is responsible for 90% of all cases of infection associated with rotavirus in the UK.

(c) Salmonellae

Salmonellae are pathogenic to man. They are gram negative, motile rods which are calalase positive, oxidase negative and faculatively anaerobic. Salmonellae are associated with bacterial food poisoning and are thus excreted in human faeces. The number that are present in sewage are heavily dependent on the health of the population served by the sewerage system in question.

(d) Pseudomonas

The differentiating characteristics of pseudomonas are that they are unicellular, non-photosynthetic, non-sporing, rod-shaped singly; motile with polar flagella or non-motile (very rare). Pseudomonas are gram negative, aerobic, heterotrophic, oxidative and produce acid only from carbohydrates.

There are many species of pseudomonas. The species investigated during the trial was *Pseudomonas aeruginosa*. At least half of the species, and possibly all of them, are found in soil or water. Ninety of the 149 recorded species are pathogenic to plants. Two species, *P. aeruginosa* and *P. pseudomallei* are animal pathogens. *P. aeruginosa* is also a plant pathogen and is very common in water and soils.

(e) Campylobacter

Campylobacter are microaerophilic, gram negative, motile curved or spiral bacteria, they are oxidase positive. The genus is divisible into two groups. Although campylobacters have aerobic metabolism, they are unable to grow in atmospheric oxygen. Growth occurs at oxygen concentrations of between 3 and 7%. Intestinal infection by camplylobacter has been recognised for some time, but only recently with improved laboratory techniques have they been recognised as the main bacterial cause of diarrhoea. The infecting organism is transmitted in faeces of infected people or other animals and so sea bathers are at risk, although the large volumes of sea water cause high dilution and so reduce risks.

(f) F+ Coliphage

Phage are a concentration of bacteriophage. Coliphage are phage which are parasitic on coliforms. The F+ coliphage attacks the F+ pilli of coliforms and so is very specific to coliforms. It occurs in large numbers in sewage and so is a good indicator of sewage pollution. F+ coliphage is through to be a possible surrogate indicator for viruses in sewage although at present tests are not fully developed and thus at present it is only a research tool.

(g) Staphylococcus aureus

These are unicellular, spherical, non-sporing, non-photosynthetic organisms which occasionally exceed 2 microns in diameter. They are arranged single, in pairs and in irregular clusters from both liquid and solid media and in addition are aerobic, heterotrophic, fermentative and gram positive.

The species investigated during the trial was *staphylococcus aureas* which is a skin pathogen and the common cause of boils, carbuncles and various other purulent diseases. It is only significant where large numbers are in contact with skin, for example, in swimming pools, but also bathing waters.

3.3.2 Sampling and analysis methods

Samples for non-routine microbiology were refrigerated and dispatched for analysis. In addition, 30% of the samples were duplicated and sent to a second laboratory, to provide some idea of the reproducibility of the analysis. The high cost of analysis precluded the duplication of all the samples. When samples were taken a neutraliser (5% sodium thiosulphate and 0.025% catalyse) was added at a rate of 10 ml l^{-1} to all samples. This eliminated any residual disinfectant and thus arrested the disinfection process. The neutraliser was added to sample bottles before the collection of the sample to ensure that disinfection was arrested at the time of sampling. The methods applied for each of the microbiological determinands by the two laboratories used during the trial were in most cases the same. Where different methods have been applied both are described.

One difference was the volume of sample requested by each laboratory. The volumes of sample sent to each of the laboratories were:

Sample type	Main laboratory (Wallace Evans)	Duplicating laboratory (Severn-Trent)
Crude sewage	10 litres	1 litre
Seawater	20 litres	10 litres

(a) Salmonella

Salmonella was enumerated using the method recommended for use in the water industry. A range of sample volumes were filtered using membrane filtration or a Hyflo superel. The resulting filter papers were each added to 100 ml of buffered peptone water and incubated at 37 °C for 24 hours. After incubation 0.1 ml of the buffered peptone was transferred into Rappaport Vassilidis broth this was incubated at 37 °C. Two sets were prepared and incubated for 24 and 48 hr respectively. This was then plated onto XLD agar and incubated for 24 hours at 37 °C. Black colonies were counted as presumptive Salmonella and confirmed using triple sugar iron agar. The "most probable number" (MPN) was calculated from recognised tables.

(b) F+ Coliphage

F+ Coliphage counts were determined using the method described by Havelaar and Hogeboom (1984). A sample of 1 ml and 1 ml of host culture was well mixed with 2.5 ml of soft trytone-glucose-yeast agar. This mixture was then poured onto the surface of a plate of TGY agar and allowed to set. The plates were then incubated for 37 °C at 24 hr.

After incubation the number of plaques present on the plates was counted. These were variable in size and were turbid. Results were reported as plaque forming units.

The host culture was a mutant *Salmonella typhimurium* (WG49). This organism is resistant to naladixic acid and Kanamycin and carries a plasmid from *E. coli* which causes it to produce *F. pili* and to ferment lactose. Therefore the host only detects coliphages which adsorb to *F. pili*.

(c) Campylobacter

Samples were concentrated using membrane filtration or the Hyflo Supercel method. Enrichment was carried out by adding the resulting membranes to brain heart infusion broth and blood. This was incubated under microaerobic condition at 43 °C and then subcultured onto Columbia agar base with antibiotic

supplement and lysed horse blood after 24 and 48 hours. The resulting plates were incubated microaerobically for 43 °C for 24 hours. Counts were confirmed using standard confirmation tests (i.e. gram staining etc.).

(d) *Pseudomonas aeruginosa*

The methods use by the two laboratories to enumerate *Pseudomonas aeruginosa* were different, both are outlined below:

1. Main laboratory; Various quantities of sample were filtered and the resulting membranes were added to 100 ml of nutrient broth. The broth was incubated at 37 °C for 24 hours and then streaked onto Pseudomonas selective media. This was then incubated for a further 24 hours at 37 °C. Typical colonies were then confirmed using API 20E (*P. aeruginosa*). MPN was calculated from recognised tables.

2. Duplicating laboratory; samples were filtered using a membrane filter which was then placed on a selective agar. This was then incubated at 37 °C, and the number of colonies formed were counted after 48 hours.

(e) *Staphylococcus aureus*

As with *Pseudomonas aeriginosa* the analytical methods used by the two laboratories to enumerate *Staphylococcus aureus* were different. Both methods are described below:

1. Main laboratory; Various quantities of sample were filtered and the resulting membranes were added to 100 ml of nutrient broth. The broth was incubated at 37 °C for 24 hours and then streaked onto Mannitol salt agar. This was then incubated for a further 24 hours at 37 °C. Typical colonies were then confirmed using API Staph. MPN was calculated from recognised tables.

2. Duplicating laboratory; Samples were filtered using a membrane filter which was then placed on a selective agar. This was then incubated at 37 °C and the number of colonies formed were counted after 48 hours.

(f) Enterovirus and Rotavirus

Concentration of viruses

There are several methods available for the concentration of entric viruses. Different methods were used by each laboratory. Both are described below:

Main laboratory; Beef extract was added to 10 litres of the sample and the pH was adjusted to 9.5. The sample was then shaken and centrifuged. The resulting pellet was discarded. The supernatant was adjusted to pH 3.5 and centrifuged again. The pellet was resuspended in buffer with the supernatant being discarded. The resuspended pellet was detoxified with dithizone in chloroform.

Duplicating laboratory; The method used was described by Morris and Waite (1980) and utilises epoxy-bound glass-fibre filter tubes in an adsorption-elution-flocculation technique.

Assay for Enterovirus

In common with all virus, enterovirus cannot replicate without a host organism. Buffalo Green Monkey Kidney (BGM) cells are used for the assay of enterovirus.

The samples were assayed using the agar overlay method on confluent monolayers of BGM cells in 75 cm^3 tissue culture flasks. The viruses in the concentrated suspension were allowed to adsorb on to the cells. The cells were then washed and 10-20 ml of the agar overlay medium is added. After the agar was set, the flask were inverted and incubated at 37 °C in the dark for up to seven days.

The agar overlay medium contains a neutral red dye which specifically stains live cells. Virus infected cells were apparent as areas in the monolayer where

the dye had not been taken up by the cells. These areas of dead cells (plaques), which correspond to the number of infectious units of virus in the sample, were counted and for each sample expressed as plaque forming units (PFU).

Assay for Rotavirus

Unlike the enterovirus group, rotavirus do not undergo complete replication in cells and therefore an immunofluorescence technique is used to look for the rotavirus antigens.

Cells derived from Rhesus monkey kidney were grown using flasks as monolayers. The cells were removed from the flasks by trypsinisation and seeded in 96-well microtitration plates. The plates were incubated for one hour and then 100 µl of sample concentrate was added to each well. The plates were centrifuged and incubated at 37 °C for one hour. The sample was then removed and replaced with fresh medium. The plates were then incubated overnight at 37 °C.

After incubation the cells were washed, fixed and air-dried. Rabbit anti-rotavirus anti-serum was added to each well and the plates are incubated for one hour at 37 °C. After washing 100 µl of FITC (Flourescin Isothiocyanate Isomer I) conjugated goat-anti-rabbit anti-serum was added to each well and the plate incubated at 37 °C for 1.5 hours. After washing the wells were examined using an inverted microscope at an excitation wavelength of 495 nm and magnification X200.

One fluorescing cell is considered to be the consequence of infection by one rotavirus particle and the number of rotaviruses present is calculated on this basis. The results are quoted in terms of fluorescing foci (FF).

4 TRIALS WITH ULTRAVIOLET RADIATION

4.1 UV disinfection systems tested at Menagwins STW

This trial was designed to study the potential of ultraviolet radiation as a sewage disinfectant. It looked at a secondary effluent which is likely to be less demanding than a screened sewage outfall. This trial is reported in detail in NRA report R&D 231/3/SW (Realey and Brogden 1991). This chapter summarises the trial and discusses its findings.

Figure 4.1 shows the layout of the Menagwins STW and the position of the UV systems for the trials. The sewage treatment process is alternating double-pass filtration. In this system the sewage effluent goes to one set of filters first for a certain period, and then the effluent is sent to the other set of beds. Thus the filters alternate between being the primary and secondary beds. Figure 4.2 shows the layout of the UV disinfection system at the site. The trial studied the performance of two systems: a low pressure UV unit and a medium pressure UV unit.

The low-pressure UV unit was a UV 2000, supplied by Sunwater Ltd and built by Trojan Technologies Incorporated, of Canada. Effluent flowed through a stainless steel channel 7.9 m long, 381 mm wide and 914 mm deep. The channel contained two UV reactor banks, each with 20 low-pressure mercury lamps, and two UV detectors which measured the intensity of UV light at 354 nm. The UV lamps in each module had a UV output of 26.7 watts, had a lifetime in the region of 17 500 hours, and were spaced 75 mm apart within each module. Ninety percent of the output of the lamps should be at 254 nm, the optimum wavelength for bacterial kill. The modules have a power consumption of 400 watts. Varying the number of banks in use (0,1 or 2), or altering the flow, affected the UV dose. The two banks of modules within the channel will treat flows between 16 - 40 litres/second. The disinfected effluent from the Trojan UV 2000 system discharged directly into the sewage works effluent channel before entering the St Austell river.

Figure 4.1 - Menagwins STW

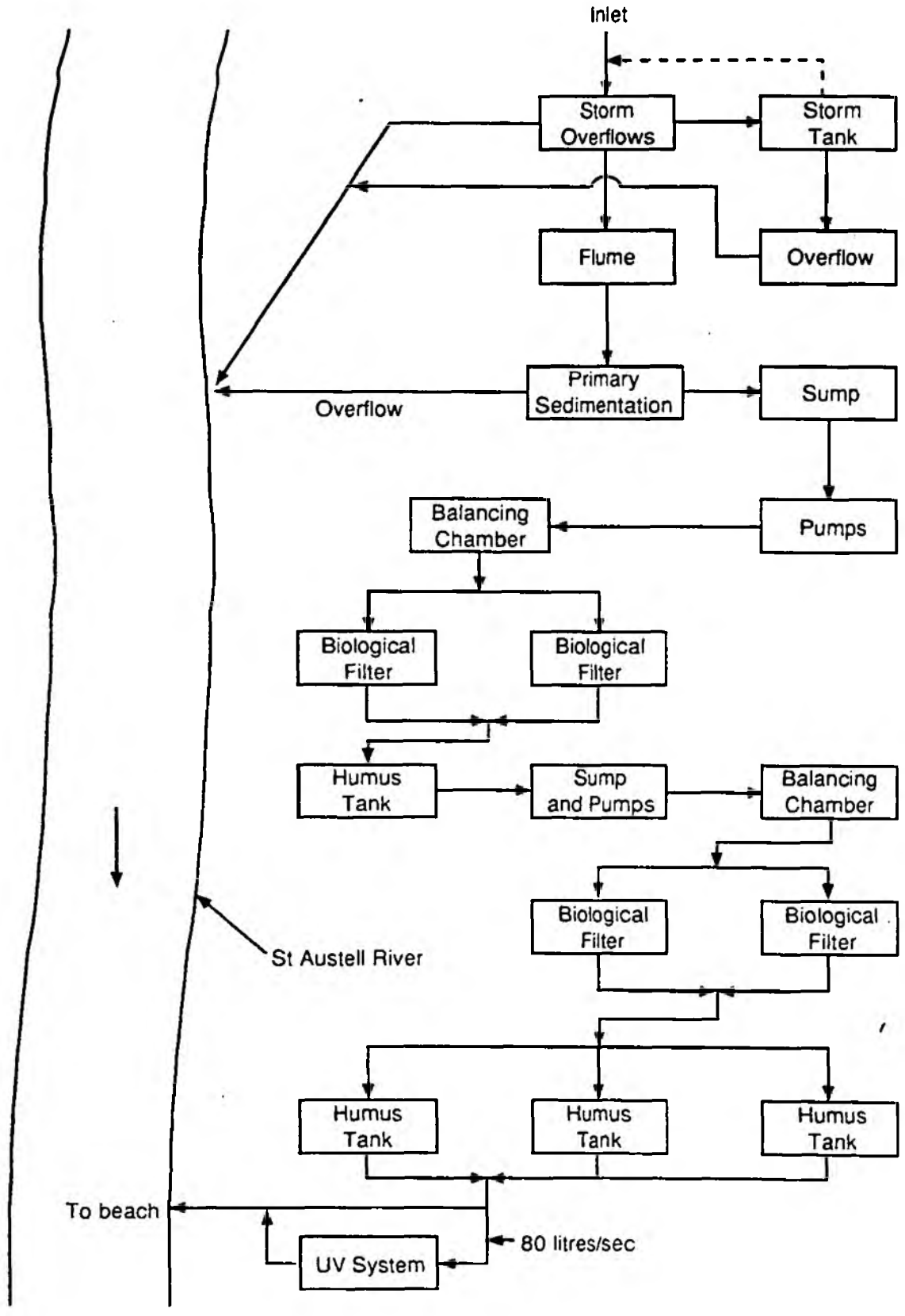
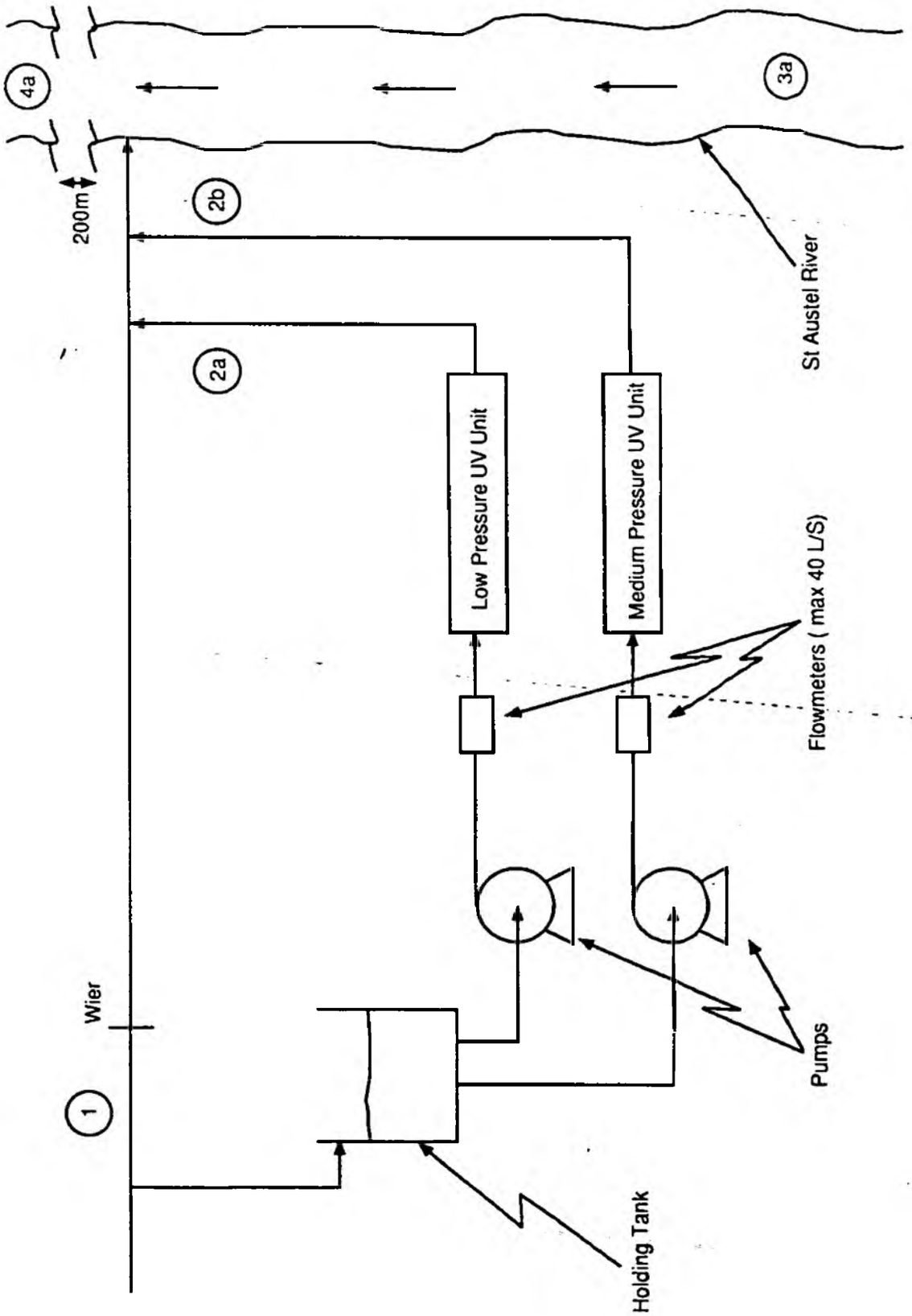


Figure 4.2 - UV Disinfection System at Menagwins STW



The medium-pressure UV unit was designed by Bronzepalm. It comprised five separate reactors mounted in parallel between common supply and discharge manifold pipes. Each reactor consisted of a stainless steel tube with a flange at each end, within this was located a single medium-pressure mercury lamp held in a quartz sleeve. Each reactor was connected to the manifold pipes by 75 mm PVC manually operated butterfly valves. This allowed for the total isolation of any reactor when not in use for cleaning or maintenance purposes. The medium-pressure mercury lamp held in each reactor had a power consumption of 5.8 kW. The majority of the discharge was at a wavelength of 254 nm.

The UV dose applied to the sewage influent to each reactor could be varied by altering the power to the lamp or adjusting the flowrate. Each reactor was capable of treating influent up to 12 litres/second, but with a design flow of 8 litres/second. Total maximum flow to the medium-pressure unit was 40 litres/second, which could be allocated evenly to each of the five reactors at 8 litres/second or less, or, some reactors could be closed and the flowrate through the remainder could be varied up to a maximum of 12 litres/second.

4.2 Aims of the Menaqwins field trials

The main aim of the study was to establish the performance of the UV disinfection systems on trial under realistic field conditions. To achieve this a number of sampling runs were performed between October 1990 and January 1991, the samples from which were analysed for the appropriate chemical and microbiological determinands. Table 4.1 lists the sampling runs and refers to the sampling points shown in Figure 4.2. The sampling points were:

Point

1 Sewage effluent before disinfection;

2a Sewage effluent after disinfection in low-pressure UV system;

2b Sewage effluent after disinfection in medium-pressure UV system;

2c Sewage effluent discharged into receiving waters;

3a Receiving waters upstream of works;

4a Receiving waters downstream of works.

The sampling runs each had specific objectives:

Run

1 Establish the quality of effluent before disinfection;

2a Establish effects of low and medium pressure UV at 50% design dose;

2b Establish effects of low and medium pressure UV at 100% design dose;

2c Establish effects of low and medium pressure UV at 150% design dose;

3&5 Study effects of systems over 24 hours;

4 Study the effects on the receiving water.

The study also considered photoreactivation and tried to answer the following questions:

(i) What is the level of photoreactivation that occurs at 3 hours exposure to sunlight for each of the organisms tested?

(ii) How does the dose of UV radiation received by the organisms affect the level of photoreactivation?

(iii) Does the photoreactivation occur once the organisms are discharged into the receiving waters?

4.3 Chemical and microbiological analysis

Chapter 3 describes the determinands and their methods of measurement. All samples for routine analysis were taken synoptically, except where large volumes of sample were required, such as for non-routine microbiology (20 litre). Photoreactivation studies required two samples. One was held in the sunlight for three hours and the second placed in a closed box for three hours. Analysis was then carried out immediately. The temperature of the effluent was low during the experiments so it is unlikely that there would have been any significant regrowth of the organisms under investigation.

Table 4.1 - Sampling runs undertaken during the UV disinfection trial at Menagwins

Sampling run number	Dates	Duration hours	UV system	Sampling points examined
1	2-3 Oct 1990	24	No	2c, 3a, 4a
2(a)	9 October 1990	8	Yes	1, 2a, 2a dark
2(b)	10 October 1990	8	Yes	2b, 2b dark
2(c)	11 October 1990	8	Yes	
3	23-24 Oct 1990	24	Yes	1, 2a, 2a dark, 2b, 2b dark
4	6-7 Nov 1990	24	Yes	1, 2a, 2a dark, 2b, 2b dark, 3a, 4a (i) (ii) (iii)
5	8-9 Jan 1991	24	Yes	1, 2a, 2a dark, 2b, 2b dark

4.4 Results and discussion

This trial yielded a mass of interesting data on the disinfection of secondary effluent by UV radiation. Pilot studies carried out at Rye Meads STW (Thames Water) during 1989 showed the potential of this form of treatment (Vincent *et al* 1991). However, the quality of the effluent at Rye Meads was extremely good and probably not representative of typical effluents from sewage treatment works. The UV transmission of the effluent at Rye Meads was around 60% and suspended solids of approximately 14 mg l^{-1}. This compares with typical UV transmission at Menagwins of between 40 and 50% and an average suspended solids value of around 14 - 27 mg l^{-1}. The level of indicator bacteria (TTC and FS) in the effluent at Menagwins was typically up to two orders of magnitude higher. The effluent at Menagwins provided a much greater challenge to the two systems under examination.

Table 4.2 summarises the results from the low-pressure system and Figures 4.3 to 4.6 show FS and TTC log reduction versus UV transmission for the low-pressure system, combining all the data from the Menagwins trials. Figure 4.3 shows a clear trend between the TTC log reduction and the UV dose.

Figure 4.3 - Low-pressure system : UV dose vs TTC reduction

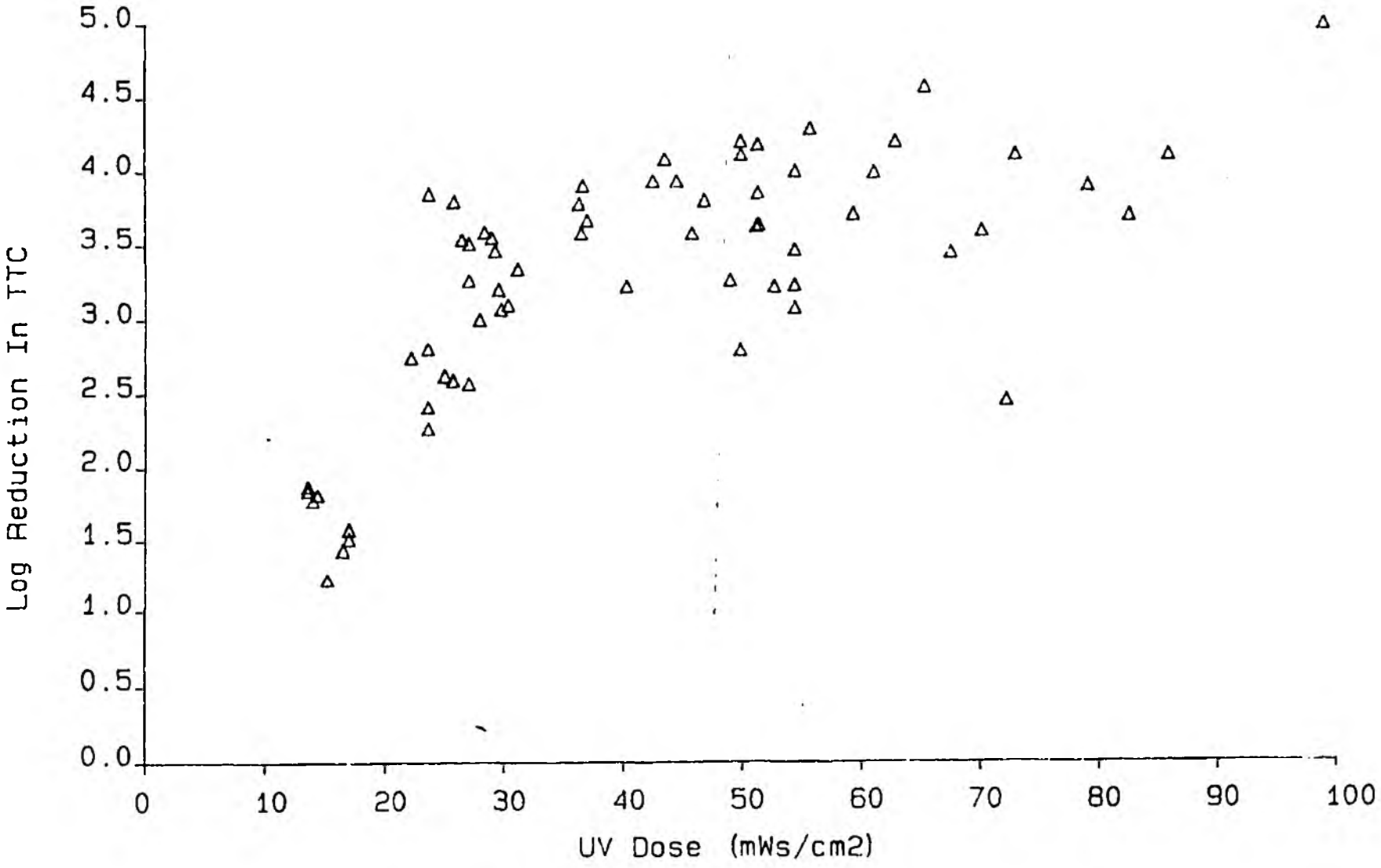


Figure 4.4 - Low-pressure UV system : UV dose vs TTC reduction after 3 hours in sunlight

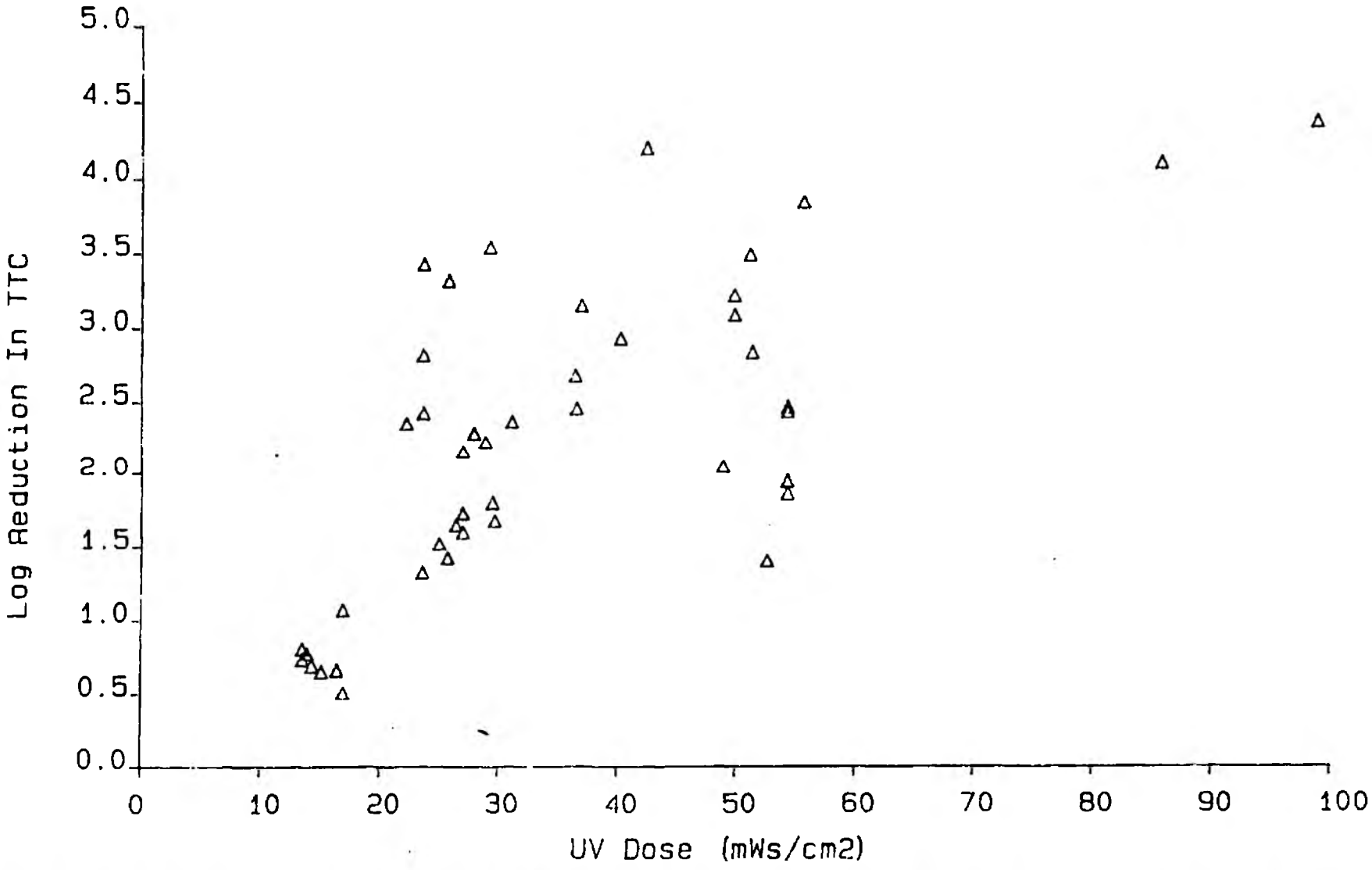


Figure 4.5 - Low-pressure UV system : UV dose vs FS reduction

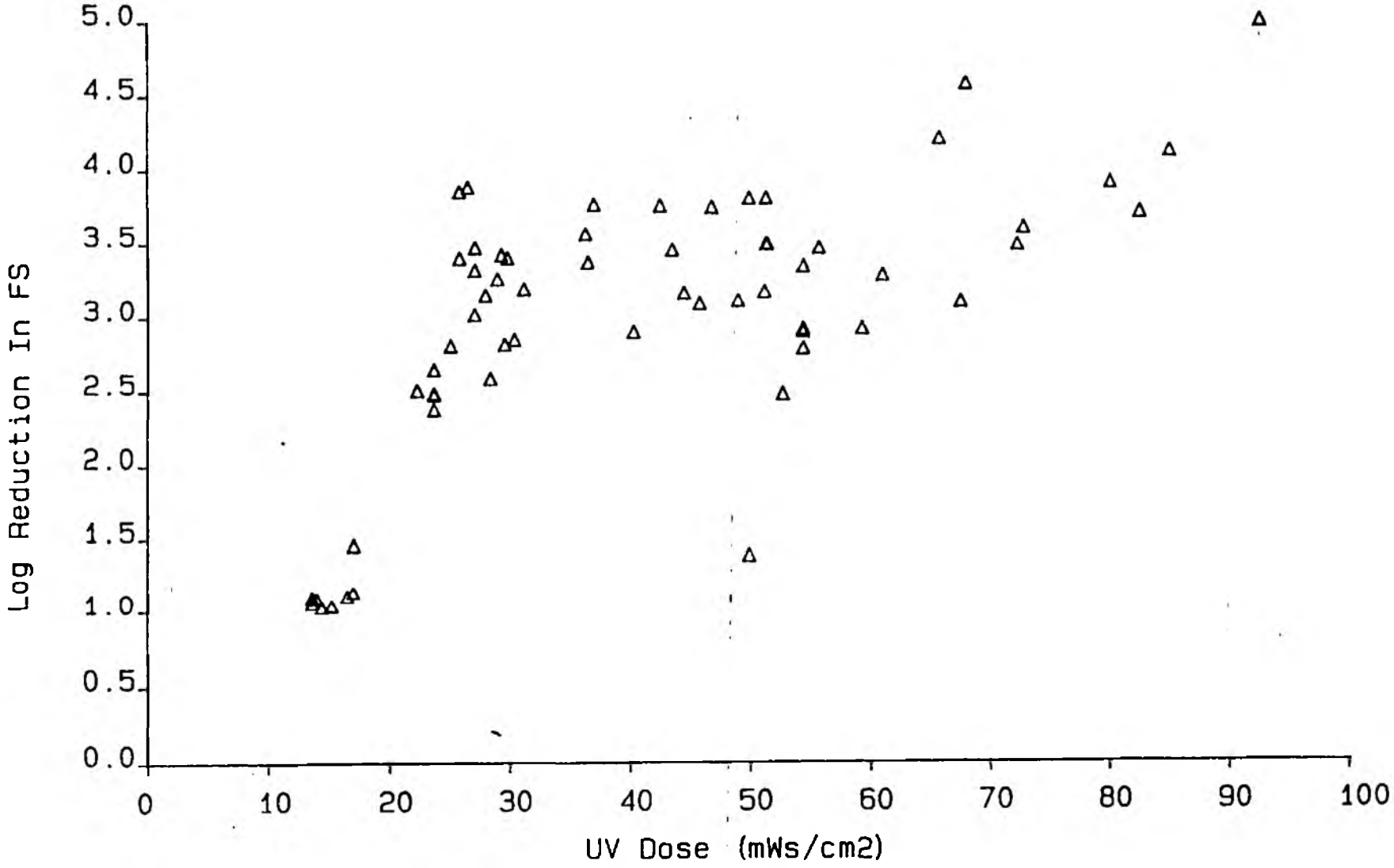
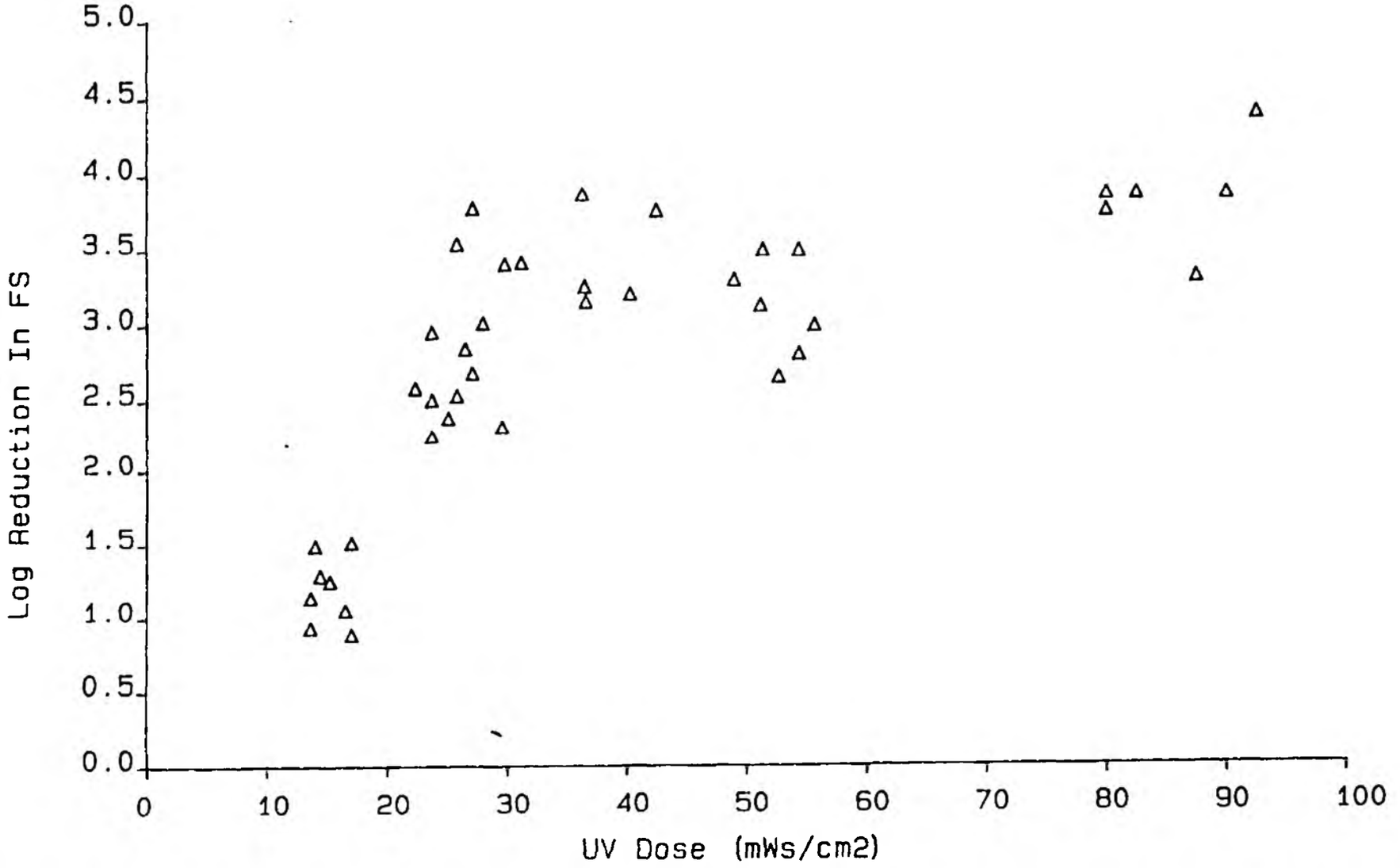


Figure 4.6 - Low-pressure UV system : Uv dose vs FS reduction
after 3 hours in sunlight



There is some scatter at the high doses, which could result from:

(i) the range of suspended solids in the effluent (this factor cannot be included in the dose calculation);

(ii) the variation in the initial number of TTC in the effluent; or

(iii) small variations in the counts at the lower end (between say, 10 - 100/100 ml) produce large variations in the log reduction.

A similar trend exists for the FS log reduction versus the UV dose. It is noticeable that the FS reduction and TTC reduction are very similar. With chemical disinfectants, such as chlorine or PAA, FS are usually found to be more resistant. In general, for those chemical disinfection systems that have been widely tested, if a three log reduction in TTC was achieved then the FS log reduction would be expected to be around 2.

Table 4.2 - Summary of results of log reduction achieved for low-pressure UV system

Parameter (mean)	Sampling points 2a	2b	2c	3	4	5
UV Dose (mWs/cm^2)	25.0	15.2	53.0	44.6	25.8	75.6
UV Transmission (%)	41	48	54	45	44	77
Suspended solids ($mg\ l^{-1}$)	28	21	17	14	27	13
Number of samples (for Photoreactivation)	9 (9)	9 (9)	9 (9)	24 (10)	5 (5)	24 (10)
TTC reduction	2.8	1.6	3.7	3.6 ()	3.4 ()	>4.2
TTC reduction after 3 hours in sunlight	2.1	0.7	2.6	2.8	2.3	>4.1
FS reduction	2.7	1.1	3.2	3.2	3.3	>3.5
FS reduction after 3 hours in sunlight	2.5	1.1	3.1	3.4	2.2	>3.3

Figure 4.4 and the results in Table 4.2 show that there was a substantial degree of photo-reactivation if the samples were held in sunlight for 3 hours.

However, the samples were held in a bottle in the sunlight, which only had to pass through a relatively short path of effluent. Therefore the intensity was higher than would normally be found in a receiving water and the degree of photo-reactivation may be higher than in a typical receiving water. The degree of photo-reactivation in these trials may represent the worst that would happen in reality.

Unfortunately adverse weather conditions hampered the attempt to measure the degree of photo-reactivation in the receiving water during this trial. It is essential that this is attempted again as it is important to establish how the receiving water will affect the degree of reactivation.

For the low-pressure system it appears that at higher applied UV doses the degree of photo-reactivation decreases. It would seem that for doses in excess of 50 - 60mWs/cm^2 the photo-reactive effect is not as significant, though the data are scattered and for some data points there has been marked photo-reactive effects even at the higher doses. This threshold is higher than quoted by the manufacturers. It may be that a lower dose would be adequate if the photo-reactive effect is not as great in a receiving water. This needs to be investigated.

The data in Table 4.2 and Figures 4.5 and 4.6 show that FS do not photo-reactivate. This concurs with the conclusion of the pilot trial in 1989.

Table 4.3 summarises the results for the medium-pressure system and Figures 4.7 to 4.10 show TTC and FS reduction versus UV dose with and without exposure to sunlight.

Figures 4.7 and 4.9 show there was a wide variation in the reduction of both TTC and FS at similar doses. This is particularly true of the lower doses, where often high kills were achieved. In general the dose required to achieve a given kill was less for the medium-pressure system compared to the low pressure system.

Figure 4.7 - Medium-pressure system : UV dose vs TTC reduction

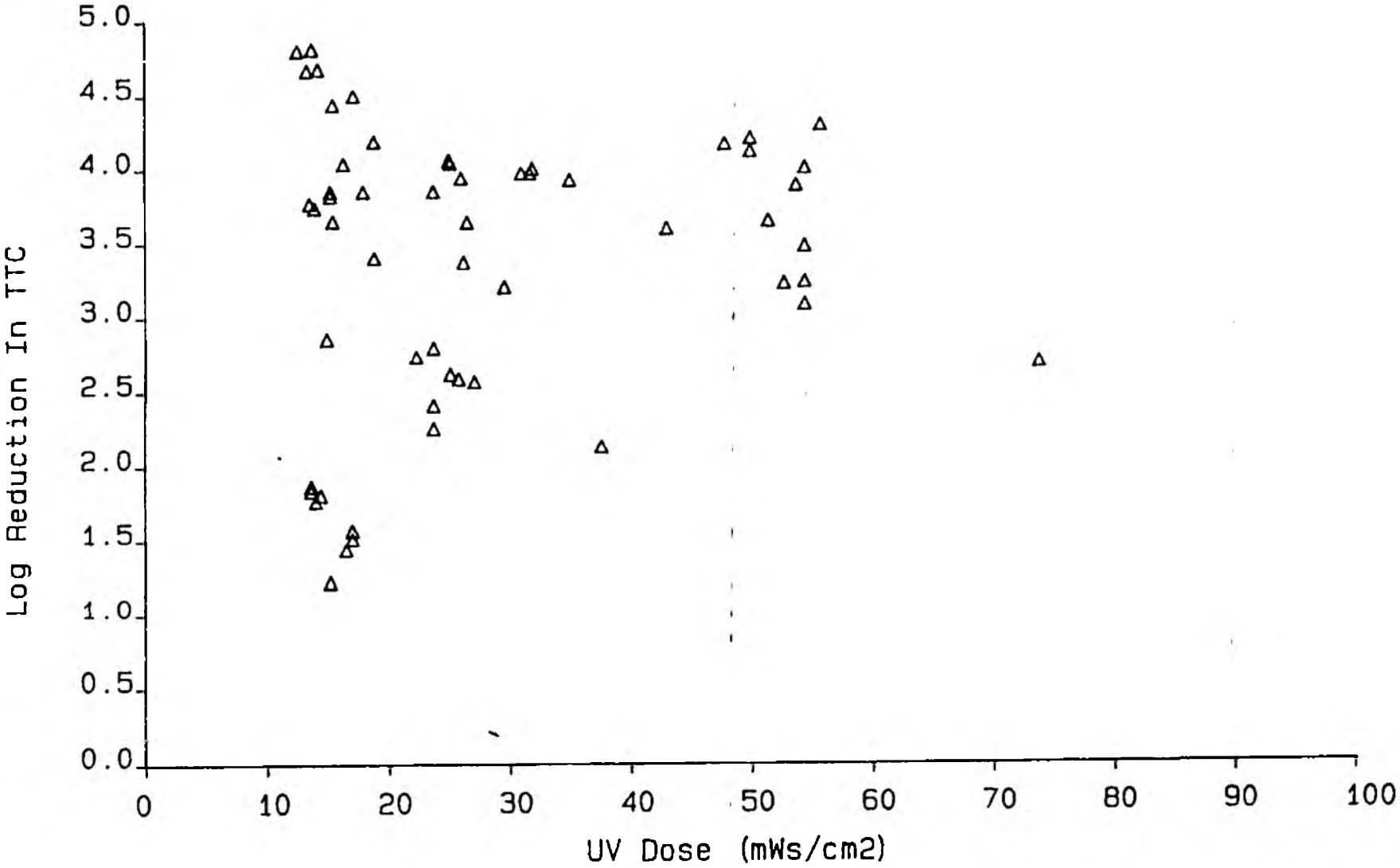


Figure 4.8 - Medium-pressure system : UV dose vs reduction in TTC after 3 hours in light

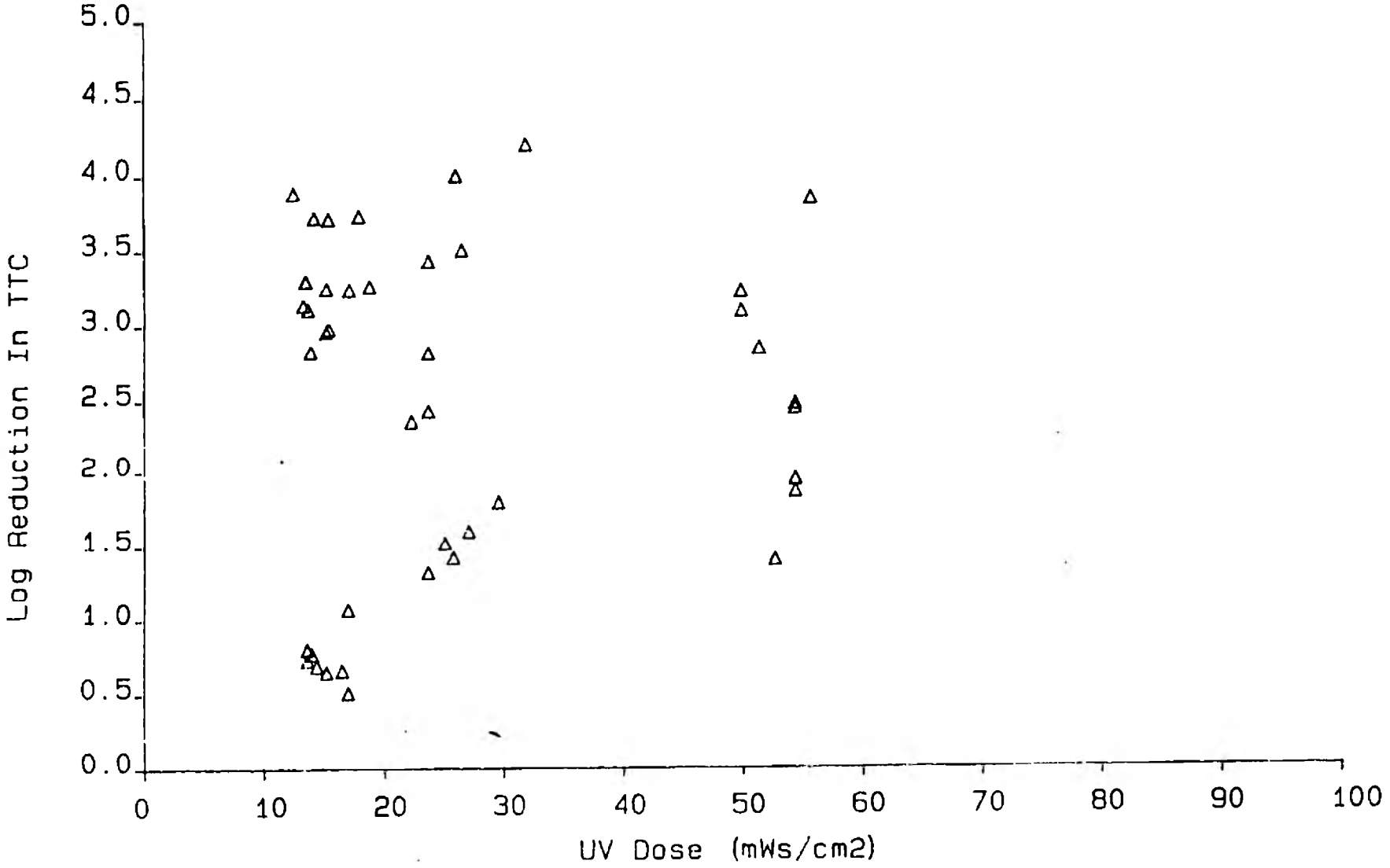


Figure 4.9 - Medium-pressure system : UV dose vs FS reduction

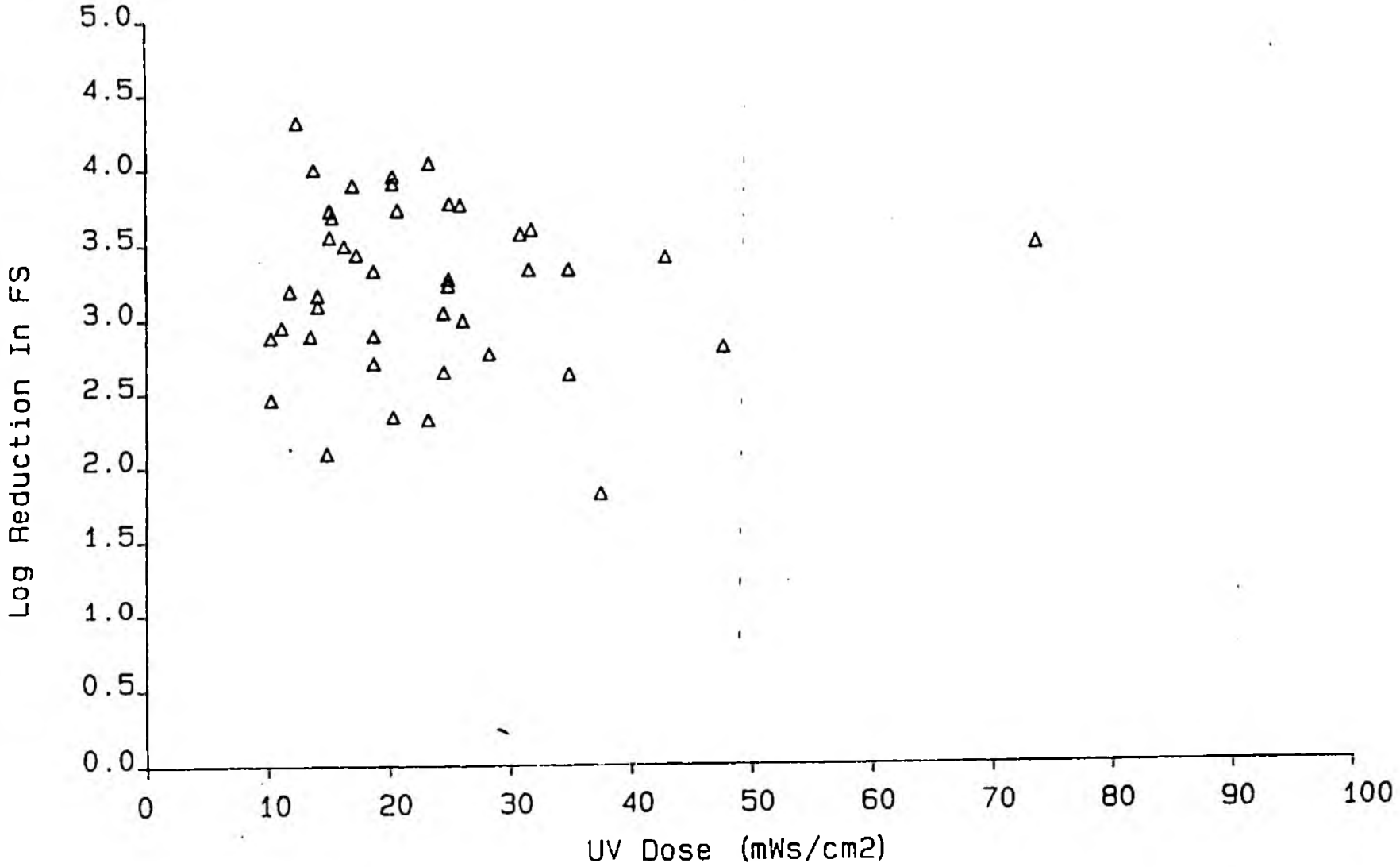


Figure 4.10 - Medium-pressure system : UV dose vs FS reduction
after 3 hours in light

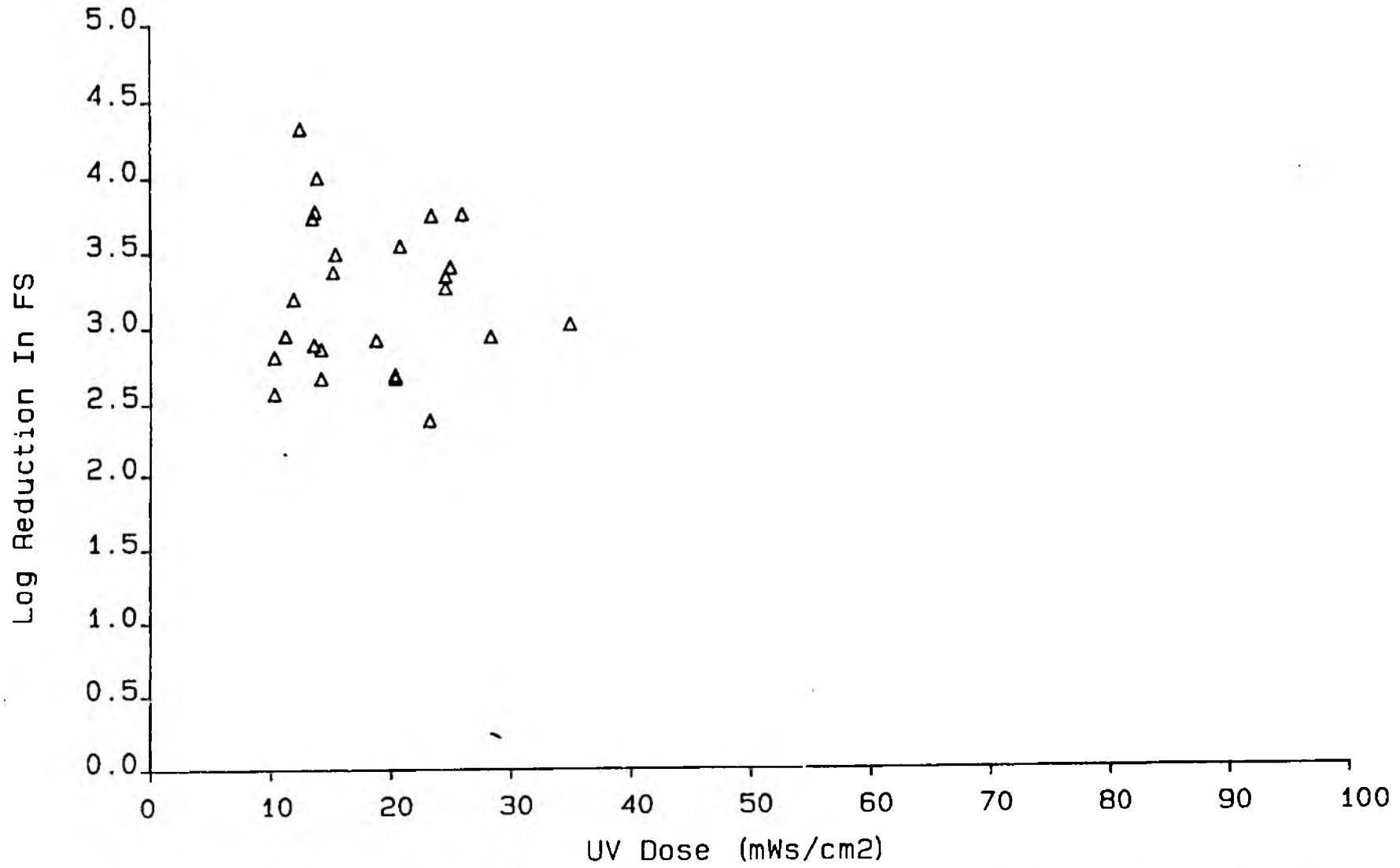


Table 4.3 - Summary of results of log reductions achieved for medium-pressure UV system

Parameter (mean)	Sampling run 2a	2b	2c	3	4	5
UV Dose (mWs/cm^2)	23.4	18.9	11.5	28.1	14.4	76.7
UV Transmission (%)	41	48	54	45	44	77
Suspended solids ($mg\ l^{-1}$)	28	21	17	14	27	13
NUMBER OF SAMPLES	9 (9)	8 (8)	5 (5)	24 (10)	5 (5)	24 (10)
TTC reduction	3.6	3.4	3.0	3.7	4.7	>4.3
TTC reduction after 3 hours in sunlight	3.2	2.0	1.8	3.4	3.5	>4.3
FS reduction	3.3	2.9	2.9	3.3	4.1	>3.3
FS reduction after 3 hours in sunlight	3.1	3.0	2.9	3.7	4.1	>3.5

As with the low-pressure system there was a significant photo-reactive effect of TTC (see Figures 4.7 and 4.8). The wide scatter of data makes drawing firm conclusions difficult. As with the low-pressure UV system there was no apparent photo-reactivation at the higher UV doses. It is not possible, however, to identify the threshold dose where the photo-reactive effect becomes insignificant. It is also not possible to say whether the bacteria treated by the medium-pressure UV systems are more or less likely to photo-reactivate than those treated by low pressure systems. FS did not photoreactivate in any of the sampling runs (see Figures 4.9 and 4.10 after treatment in the medium pressure UV system).

One possible reason for the scatter in the TTC and FS reduction at the lower doses is the flow pattern in the medium-pressure UV system. The dose was varied by changing the flowrate through the system. At low flows a laminar flow pattern may have developed. Bacteria near the wall of the reactor might not have received a very high dose of UV radiation. This would have caused low apparent kill. Ideally a flow pattern should exist along the axis of the bulb with turbulence across the phase of the reactor, as happens when the flow is higher but the dose is low due to low UV transmission.

In general the results from the non-routine microbiological analysis were disappointing because of the large numbers of low and indeterminate counts. Chapter 7 discusses these in detail for all the trials. It is therefore difficult to drawn any firm conclusion from the data. There were considerable differences between results from the two laboratories. The main laboratory consistently found larger numbers of F^+ Coliphage than the duplicating laboratory. The duplicating laboratory tended to recover large numbers of Enterovirus at sample point 1 while the counts carried out by the main laboratory yielded few Enterovirus. The difference may have been due to the different analytical methods applied by the two laboratories.

The methods used for the analysis are those developed for the food industry or for clinical examinations and they have not undergone extensive development or evaluation for environmental use, where interferences may be different. A pre-enrichment step may allow environmentally stressed organisms to recover. In environmental samples, where there are complex interactions, the time delay between sampling and examination may have considerable influence on the numerical value of the result.

It is not possible to link the reduction in each of the non-routine micro-organisms to the UV dose applied. This area needs further work. However, the results from both laboratories indicate that UV radiation was reducing the levels of *Staphylococcus aureus, Pseudomonas aeriginosa* and F^+ Coliphage. The effect on enterovirus is not clear: results from the main laboratory suggest that UV is having little effect, but the data from the duplicating laboratory imply that enterovirus numbers are being substantially reduced. Results from the duplicating laboratory also implied that UV radiation was effective against Salmonella. This was not backed up by the main laboratory. Clearly more work is needed in this area.

In order for a disinfection technique to be useful it must be demonstrated that it can reduce the levels of pathogenic organisms. The data gathered in this trial hinted that UV radiation will meet this criterion but they are not conclusive.

The low-pressure UV system was much more robust from a practical and operational viewpoint. Cleaning the system was easy and can be accomplished by a single person. The bulbs are lifted from their channel (after they have been turned off) and are wiped with a cloth soaked in dilute acid. This system at Menagwins took one-man-hour to clean. The medium-pressure system was difficult to clean and the inner quartz sleeve was easily broken. The installation at Menagwins took one man-day to clean and was a job for two people. The medium-pressure system also heats up very quickly and will 'trip out' once the temperature reaches a high level. It does not turn on once the flow has returned. Some further automation is therefore required if the system is to be run continuously. It was not established how often the lamps will need cleaning or how long the lamp will last. This will require investigation if operating costs are to be established.

4.5 Conclusions on the use of ultraviolet light to disinfect sewage effluent

1. Conclusions drawn from this work can be directly applied to sewage works effluent produced by biological filtration. Other biological treatment systems such as activated sludge produce different types of solid particle. This may affect the treatment efficiency of the UV disinfection plant.

2. Both the low- and medium-pressure UV systems proved successful at significantly reducing the numbers of thermotolerant coliforms (TTC) and faecal streptococci (FS).

3. Both UV systems reduced the level of TTC by three orders of magnitude (99.9%) providing a large enough UV dose was applied.

4. The medium-pressure system required a lower dose to produce the same level of TTC and FS reduction.

5. After three hours in sunlight TTC significantly photoreactivated. This photoreactivation occurred with both systems and was related to UV dose. It did not occur at the higher UV doses.

6. Photoreactivation of TTC did not occur once the UV dose passed above a certain threshold. This threshold dose was not identified precisely by this work but falls in the range 50 - 75 mWs/cm^2 for both UV systems.

7. The method used to assess photoreactivation was designed to produce the "worst case" effect. This trial did not establish whether photoreactivation of TTC would occur in a receiving water.

8. The level of reduction in FS was found to be similar to that of TTC for both UV systems.

9. There was no evidence that FS was subject to photoreactivation after treatment in either of the UV systems.

10. Both UV systems reduced the levels of F$^+$ Coliphage, *Staphylococcus aureus* and *Pseudomonas aeriginosa*. Results of enterovirus, rotavirus, Salmonella and Campylobacter analysis were inconclusive.

11. There was no change in BOD, COD, ammoniacal-nitrogen, TON, phosphorus, chloride, or pH as a result of UV disinfection.

12. The UV transmission decreased as a result of UV treatment. The level of decrease was found to be proportional to the UV dose.

13. The low-pressure UV system used less power than the medium-pressure UV system to produce the same UV dose.

14. The low-pressure system took approximately 1 man-hour to clean and was generally the more robust of the two systems.

15. The medium-pressure system took approximately 1 man-day to clean. The quartz sleeves were very vulnerable to breakage.

16. Overall, the UV radiation looked to be a promising method for disinfecting secondary effluent.

4.6 Data gaps

This trial identified several areas in which further work would be desirable.

1. The threshold UV dose above which no photoreactivation occurs needs to be more closely defined.

2. More work is required to identify the level of reduction achieved in non-routine organisms by UV disinfection. Viruses are of particular concern.

3. Information is required to establish whether TTC and other organisms are subject to photoreactivation once in a receiving water.

4. The following questions must be addressed before operating cost can be estimated.

   (i) What is the lamp life?
   (ii) How often do the UV systems require cleaning?

5. TRIALS WITH PERACETIC ACID AND FINE SCREENED SEWAGE

5.1 PAA dosing at Trevaunance Cove

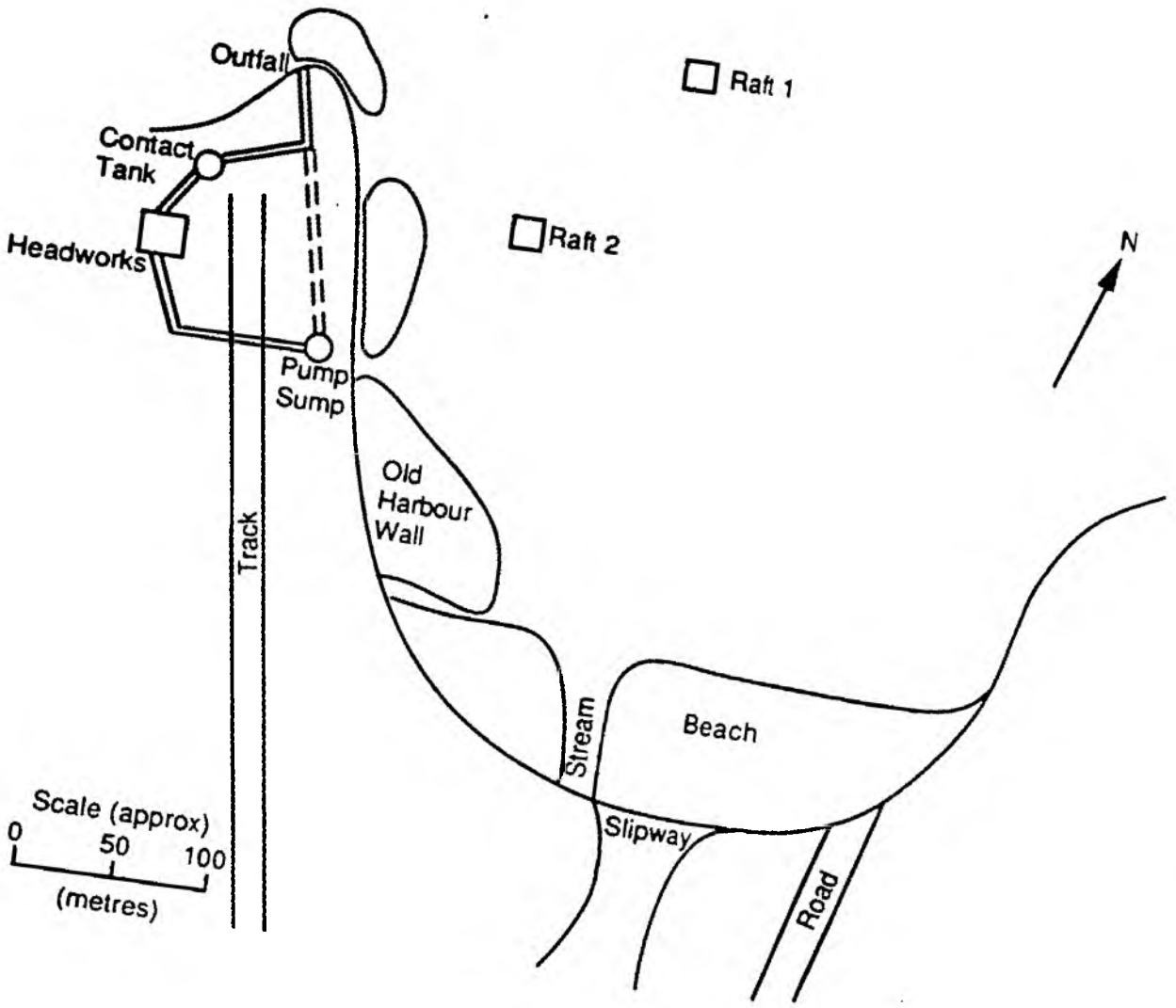
This chapter describes disinfection trials using PAA with fine screened sewage from Trevaunance Cove marine headworks that discharges fine-screened crude sewage to the sea via a short sea outfall at Trevaunance Cove, St Agnes, Cornwall. These trials built on the initial field and laboratory work on PAA and sewage described in Section 2.3, which highlighted the possibility that the organic content of the sewage exerted a significant demand on the oxidising powers of PAA. NRA interim report R&D 231/2/SW (Roddie *et al* 1991) describes this trial in detail and this chapter summarises the work and discusses the findings.

Figure 5.1 shows the position of the works in relation to the beach and surrounding area. The headworks serves most of St Agnes. The source is wholly residential with all the sewage being pumped to the works. The estimated residential population being served is 2400, with an average dry weather flow of 430.4 m^3 day^{-1} . This rises in summer to a population of approximately 4000 as a result of tourism, which increases influent to the works.

Flows of up to 53 litres/second pass to the headworks for treatment. Flows in excess of this pass via an overflow from the pump sump directly to the outfall. Flows of up to 3 dry weather flow (DWF) are pumped to the headworks by means of two pumps which are operated by trigger levels when the sump is full. One pump operates up to 18 litres/second with the second storm pump operating to assist above this level up to 53 litres/second.

Influent flow passes through a Polcon microstrainer fine screen (with wedge wire apertures of 1.5 mm), that operates intermittently due to the pumped nature of the influent. Screenings from this are automatically discharged into a skip which is housed within the headworks building. The screened sewage then passes into a collection trough beneath the rotostrainer and flows to a holding tank, of approximate 7 m^3 volume, prior to discharge to the outfall. This holding tank allows a retention time before discharge to the sea through an outfall pipe approximately 25 m in length.

Figure 5.1 Trevaunance Cove Headworks and Location



The effluent from the headworks discharges into the sea to the west of Trevaunance Cove. This creates a plume, which is sometimes visible, the position and extent of which varies according to wind, wave and tidal state. The beach has a north westerly aspect and is exposed to the prevailing wind. Consequently it may become polluted by sewage entering the cove (see Figure 5.1).

Peracetic acid was pumped from the specially designed tanks of 1000 litres capacity by means of a stainless steel bodied pump fitted with a PTFE diaphragm and seals. These are the only materials which are suitable for long term exposure to peracetic acid. There were two dosing pumps each comprising of a liquid pumping end, pump drive mechanism and a manual stroke positioner, all mounted on a common baseplate. The drive was provided by an electric motor.

While one pump was on-line, the other acted as standby. Prior to each pump there was an open/close valve and after each a pressure release valve to prevent the system from over-pressurising. The overflow from these discharged within the headworks building.

Peracetic acid was dosed to the screened sewage in a collection trough beneath the Polcon rotostrainer. The screened sewage then flowed into a retention tank, which allowed a contact time for the peracetic acid to act prior to discharge to the outfall.

5.2 Aims of the Trevaunance Cove field trials

The aim of the trials was to establish the performance of PAA with screened sewage under realistic field conditions. The study was conducted in three phases. All surveys were based on 24 hour sampling sequences, during which samples were collected at a number of points with a frequency dependent primarily on the unit cost of analysis.

Each series of samples was intended to obtain data representative of the range of sewage strength and flow rate occurring over a diurnal cycle, on the necessary assumption that the 24 hour periods chosen were typical diurnal

cycles, and that day-to-day variation was not sufficient to invalidate the concept of a typical cycle. It may be difficult to compare the data from one series of samples with that from another, because of the variation which may occur in sewage quality over longer periods of time. No rainfall occurred during any of the 24 hour sampling runs and therefore sewage quality was not affected by rain water.

Initially, a baseline survey was carried out to assess the microbiological and chemical composition of the discharge prior to disinfection. This was followed by a rangefinding trial, during which the efficacy of different PAA dose rates was investigated. A fixed dose regime was established on the basis of the results of the rangefinding trial. PAA was administered at this fixed dose regime for the remainder of the study period, and microbiological, biological and chemical effects were monitored during a series of 24 hour sampling sequences.

Standard sampling points were established at the beginning of the study (Figure 5.2). Table 5.1 indicates the nature of the sample obtained at each point.

Table 5.1 - Sampling points and nature of sample

Point	Nature of sample
1	Crude sewage prior to screening and contact tank
2	Crude sewage after screening and contact tank
4a	Centre of sewage plume approx. 20 m from point discharge to sea (surface sample)
4b	Edge of sewage plume approx. 200 m from point of discharge than 4a (surface sample)

Figure 5.2 Trevaunace cove treatment works

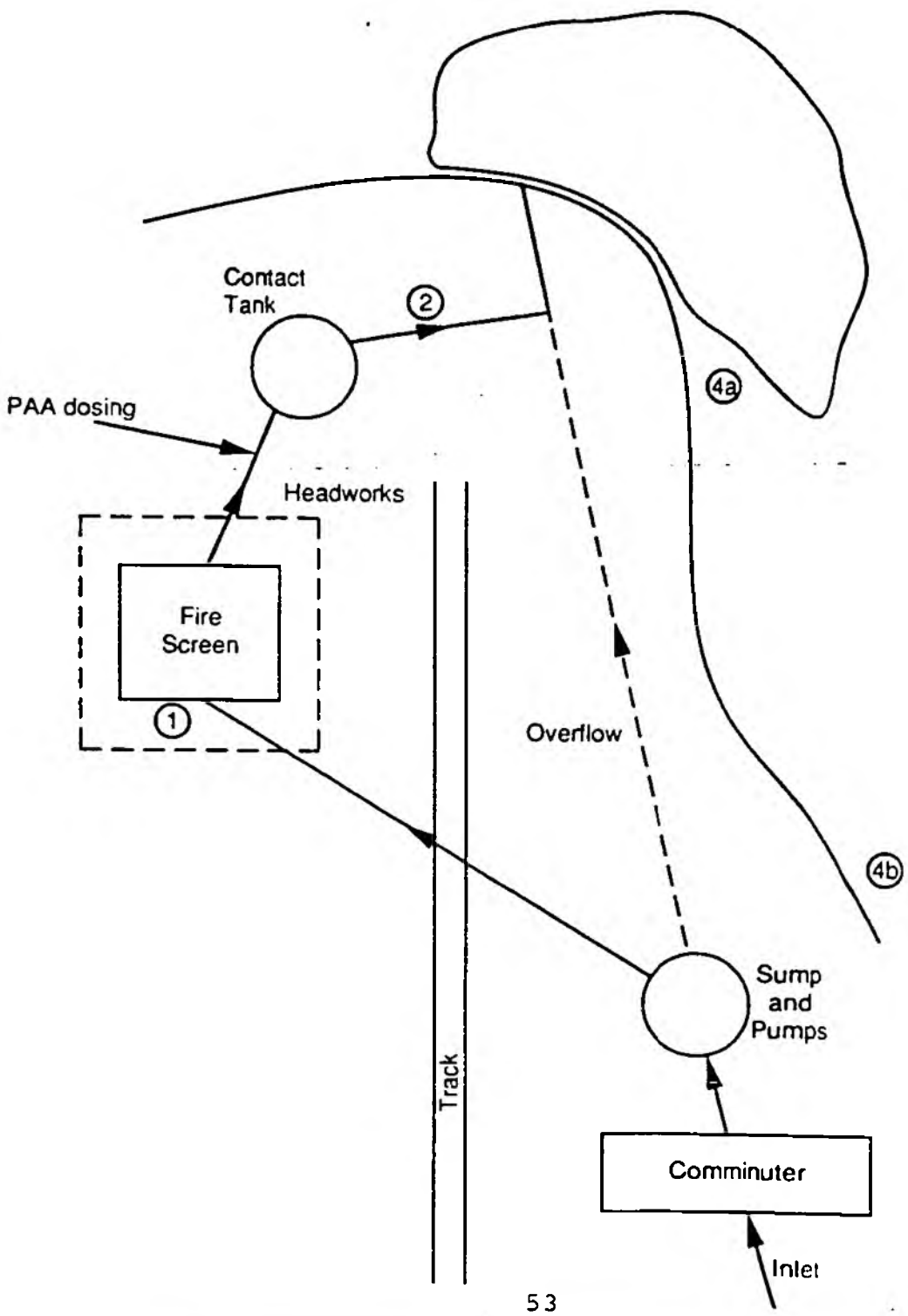


Table 5.2 summarises the timing, duration and dose level of each of the trials. The baseline trial consisted of a single 8 hour series of samples on 17 and 18 July 1990.

Table 5.2. Details of sampling runs undertaken during the trial

Sampling run number	Dates	Duration (hours)	Peracetic acid dose (mg l^{-1})	Sampling points examined
1	17-18 July 1990	8	0	1, 2, 4(a), 4(b)
2(a)	31 July 1990	8	12	1, 2
2(b)	1 August 1990	8	14	1, 2
2(c)	2 August 1990	8	16	1, 2
3	14-15 August 1990	24	20	1, 2, 4(a), 4(b)
3(a)	5-6 September 1990	16	20	1, 2, 4(a), 4(b)
4	21-22 August 1990	24	20	1, 2
5	11-12 September 1990	24	20	1, 2, 4(a), 4(b)

The dose determination trial comprised a 24 hour series at different dose levels (12, 14 and 16 mg l^{-1} PAA) on three consecutive days between 31 July and 2 August 1990.

The fixed dose trials (20 mg l^{-1} PAA) were conducted over 24 hour periods on four dates between 14 August 1990 and 12 September 1990. Interox were asked to set the dose of peracetic acid to achieve the required reduction in TTC. They carried out some spot tests and concluded that a dose of 12 mg l^{-1} peracetic acid would be adequate. Spot tests carried out by WRc earlier in 1990 indicated that a higher dose would be necessary (14-16 mg l^{-1} peracetic acid). These spot tests were carried out by taking samples of the sewage from Trevaunance Cove and adding the peracetic acid using a syringe. The disinfected sample was then left for 10 minutes and the disinfection was stopped using a dose of neutraliser. It was decided that the first day's dosing would be at 12 mg l^{-1} peracetic acid with the second at 14 mg l^{-1} peracetic acid. If 14 mg l^{-1} was found not to be adequate to produce the 99.9% reduction in TTC, then a dose of 16 mg l^{-1} peracetic acid would be tried on the

third day. If 12 mg l^{-1} peracetic acid was enough, the dose of peracetic acid would be dropped to 10 mg l^{-1} peracetic acid.

Once the dose was established it was intended that the system would be left dosing all the time until the trial was over.

5.3 Frequency and timing of sampling

The interposition of the contact tank in the sewage flow, and the mixing of sewage within the tank, meant that a simple time lag in sampling could not be applied between sample points 1 and 2. Consequently, samples were taken (where nominal collection times coincided) approximately simultaneously.

As the flow through the outfall was intermittent, samples at point 2 were taken only when sewage was flowing, 5 minutes after flow commenced. This permitted settled material to be flushed out, and allowed sewage which had received a longer than average contact time to be eliminated from the system. The delay also ensured that NRA personnel were able to collect comparable simultaneous samples of the effluent plume at points 4a and 4b.

Samples for routine chemistry and microbiology were taken most frequently, whilst those for non-routine microbiology and chemistry were taken at times chosen to reflect a range of sewage quality. Samples for GCMS analysis and toxicity testing were taken least frequently.

Sampling was not synoptic for all determinands at a given point and time. Routine chemical and microbiological analyses were carried out on independent samples, as were non-routine microbiological analyses. Non-routine inorganic and organic chemical analyses, and toxicity tests, (where these coincided) were all carried out on subsamples of a single sample, but were not determined synoptically with any other determinands.

In conjunction with each of the five 24 h sample series, NRA staff collected data on temperature, salinity and dissolved oxygen at points 4a and 4b, in the centre and on the edge of the discharge plume respectively. Five or six samples were collected during each period.

5.4 Sewage dispersion study

Prior to undertaking the disinfection trial, it was considered necessary to gain some information on localised water movements affecting the outfall site. Consequently, a series of fluorescein dye-tracking studies were carried out on 9 July 1990.

At approximately 1.5 hour intervals, 500 ml of concentrated fluorescein solution was added to the effluent from the fine-screening plant. Observations were made (including video and still photography) from the cliff-top for as long as each dye patch remained visible. Wind speed and direction were recorded immediately after each drop.

5.5 Parameters investigated and analysis

Chapter 3 describes chemical and microbiological parameters studied and their methods of measurement. In addition, three other biological investigations were conducted: a study of the accumulation of thermotolerant coliform bacteria and F+ coliphage in transplanted and indigenous mussels was undertaken by MAFF personnel, the toxicity of sewage and receiving water samples to oyster embryos and to Microtox bacteria was assessed by WRc personnel, and field studies of rocky-shore invertebrates were conducted by NRA personnel.

5.5.1 Toxicity assessment

Ecotoxicological trials were conducted at Trevaunance Cove on 15 August 1990 and 12 September 1990. Each trial involved collecting 1 litre volumes of screened sewage prior to disinfection (point 1) and at a point after disinfection (point 2). The samples were taken during periods of low and peak sewage flow which had been previously established as approximately 0600 h and 0900 h. However, since sewage is only discharged from the pumping station when the sewage collection tank is full, the actual discharge times were approximately 0645 and 0945.

In order to obtain representative samples of disinfected sewage, these samples were collected from point 2, approximately 10 minutes after sewage had begun passing into the disinfectant mixing tank.

One litre volumes of seawater were collected in clean polyethylene containers at points 4a and 4b.

Immediately after collection each sample of sewage and seawater was filtered through a combination of coarse (GFC), 5 µm and 0.45 µm pore-size membrane filters. Filtration was necessary in order to facilitate microscopic assessment of oyster larvae at the end of the test. In the case of the Microtox test particulate matter would cause turbidity and reduce the ability of the Microtox analyser to detect bioluminescence.

(a) Oyster embryo-larval test method

For the oyster embryo-larval toxicity test, filtered sewage was then diluted to 0.032, 0.1, 0.32, 1, 3.2, 10 and 20% sewage in reference seawater. The concentrations were prepared individually in duplicate 30 ml glass test vessels. A 20% reduction in salinity has been shown by the author to have no significantly adverse effect on embryo-larval development.

Reference seawater was taken from a relatively clean offshore site near the Eddystone Lighthouse, Plymouth. This water, used as a standard in all WRc oyster embryo-larval studies, was stored in a closed black tank at ambient temperature and filtered to 0.45 µm before use.

Thirty millilitre aliquots of the seawater samples from points 4A and 4B were poured into each of three glass test vessels per seawater sample or sewage concentration. Triplicate 30 ml aliquots of reference seawater were used as controls in the oyster embryo-larval tests.

Mature "conditioned" oysters were opened by cutting the adductor muscle with a large flat-bladed scalpel. The body cavities were washed thoroughly with seawater and the gonads carefully incised so that the gametes could be removed. Reference seawater was pipetted over the gonadal surfaces in order to collect the gametes.

Egg suspensions were made up from three females (in 100 ml of seawater) and were filtered through 100 µm mesh to remove any tissue debris. Motile, mature sperm was selected from a single male and filtered through a 60 µm mesh to remove any tissue debris. Ten millilitres of sperm suspension was added to each egg suspension.

After two hours the fertilised eggs had reached the 16-32 cell stage of development and they were then microscopically examined in order to establish their quality and counted using a Sedgewick-Rafter cell. The embryo suspension was then adjusted to 1200 embryos per ml using reference seawater.

Each of the test concentrations were then inoculated with 0.5 ml of embryo suspension in order to produce an initial density of approximately 20 embryos per ml. The embryos were then incubated at 25 °C for 24 hours in darkness. After this period, 0.5 ml of buffered 20% formaldehyde was added to each test vessel to preserve the samples for subsequent counting of normal and abnormal larvae.

The numbers of normal D-shaped larvae in each duplicate dilution were expressed as percentages of total surviving larvae per 5 ml. Abbott's formula (Finney 1971) was used to correct treatment percentage survival for control mortality. Twenty-four hour EC50 values were calculated using the "moving average method" (Stephan 1977).

(b) Microtox method

Sewage samples were tested according to the standard operating procedure described by Butler (1990). Seawater samples were tested using the "100% test" methodology described by Microbics' Corporation (1989). For each set of sewage and seawater samples, collected at a particular time, a single vial of freeze-dried microtox bacteria was reconstituted.

Non-disinfected and disinfected sewage samples for each given time were tested simultaneously, using the same vial and age of bacteria. Sample holding time and temperature changes were minimal and consistent between pairs of samples.

Samples of filtered sewage for the microtox tests were prepared as serial dilutions in the range of 11.4, 22.8 45.5 and 91% sewage in 2% sodium chloride solution. During the microtox test procedure the range of concentrations are diluted by 50% and therefore tested at 5.7, 11.4, 22.8 and 45.5% sewage. Each concentration was tested in duplicate cuvettes as described in the microtox toxicity test standard operating procedure by Butler (1990). The seawater samples were tested in duplicate as 50 and 100% concentrations in reference seawater. Duplicate cuvettes of reference seawater were used as controls.

A multi-dispensing micropipette was used to add 10 µl of reconstituted bacteria to eighteen cuvettes containing 0.5 ml of precooled diluent (2% NaCl at 15 °C). Initial light levels were determined (I0). Immediately after measuring I0 values, 0.5 ml of pre-cooled serially diluted sample (ref 2.2) was added to each cuvette. Light levels (I15) were measured 15 minutes after sample transfer.

5.5.2 Microbiological contamination in shellfish

Mussels in wide mesh bags were deployed from rafts at two sites in Trevaunance Cove (Figure 5.1), and also in weighted bags placed in the old harbour area. Small naturally-occurrring mussels from a site close to the discharge were also examined. Mussels were deployed for 24 hours and then collected and analysed over a 10 day period prior to disinfection. Fresh mussels were deployed before analysis over an 8 day period following disinfection. Harbour and naturally-occurring mussels were collected at low water. Mussels and water samples from rafts were collected at high water.

E. coli counts in mussels were analysed using the method described by West and Coleman (1986). *E. coli* counts in water were performed by the membrane filtration method and F^+ bacteriophage was detected using the WG49 engineered *Salmonella typhimurium* host. *E. coli* and bacteriophage titres were expressed per 100 g of shellfish flesh and fluid or per 100 ml of water.

5.5.3 Survey of rocky-shore communities

Prior to the commencement of the disinfection trials, sets of five 0.25 m^2 observation quadrats were established adjacent to the point of discharge at Trevaunance Cove and at a reference site at Trevellas Porth.

Quadrat positions were selected at random on the rock surface at the height of the outfall pipe for the Trevaunance Cove site, and at the equivalent height for the control site at Trevellas Porth. Biological zone indicator species were used to confirm that the sites were of comparable height, and this was further confirmed with a 0.5 m cross-staff during a later visit. Heights (relative to chart datum) were 4.8 m at the outfall and 4.9 m at Trevellas Porth.

Algae, lichens and 'mat'-forming animals were assessed in terms of percentage cover, and mobile animals by density per m^2 or dm^2 as appropriate. Where organisms were assessed per dm^2, three counts were made and the average recorded. On subsequent visits the same three decimetre squares were assessed.

Specimens of organisms which could not be identified in the field were placed in a solution of 95% ethanol:5% glycerol and returned to the laboratory for examination and identification.

An attempt was made to repeat the survey on 13 September 1990, following the cessation of the disinfection trials, but inclement weather prevented completion. A complete post-disinfection survey was accomplished on 12 October.

5.6 Results and discussion

5.6.1 Sewage characteristics during trials

Flow rate followed a consistent diurnal cycle during all sampling sequences. There was, however, evidence of considerable variability during the early morning of 15 August. This contrasted sharply with a pattern of uniformly low flow at this time on other dates.

(a) Chemistry

BOD, COD, NH_3 and suspended solids levels and patterns of variation in influent sewage were broadly similar for all sampling periods. Concentrations during the 21/22 August sampling run (run 4) exhibited less short-term variability than during other sampling periods. BOD and COD loads were lower during 17/18 July (0 mg PAA/l) and 31 July-2 August (dose determination trials, 12, 14 and 16 mg PAA/l) than during later periods.

Values of pH varied less markedly over each 24 h period than did other variables.

In general, sewage was of a reasonably uniform quality in terms of basic (routine) chemical characteristics on all sampling occasions during the trials.

The concentrations of heavy metals and other inorganic determinands in influent sewage were very similar between fixed dose trials conducted on 14/15 August and 11/12 September. Values measured during the baseline trial on 17/18 July were similar to the other trials in respect of most determinands, but differed in respect of Cu, Fe, Al and Mn; these were, respectively, factors of 1.5, 10, 4 and 3 higher than in later trials. Pb levels measured during the baseline trial were approximately twice the levels measured on 11/12 September, but close to those observed on 14/15 August.

Heavy metals tended to be more strongly associated with the particulate phase during periods of high flow, and to be present in lower concentrations during periods of low flow than of high flow.

No residual oxidants were detected in influent sewage on 15 August or 12 September, the only two dates for which data are available.

Of the range of VOX and phenolic compounds for which analysis was carried out, only chloroform ($CHCl_3$) and phenol were consistently detected in the influent sewage on all sampling dates. Both compounds showed some evidence of diurnal variation in concentration, with levels being lowest during low flow periods.

(b) Microbiology

TTC numbers were similar between all sampling periods, but showed least variability during the baseline (17/18 July) and dose determination (31 July-2 August) and greatest variability during fixed dose (20 mg PAA/l) trials on 14/15 August and 11/12 September. The diurnal pattern of variation was consistent between dates. Numbers and patterns of variation of faecal streptococci were similarly consistent.

In general, the ratio of TTC to FS might be expected to be fairly constant in domestic sewage. In isolated instances (e.g. 15.55, 17 July; 04.10 18 July), single peaks in TTC numbers were observed which were not reflected in FS numbers. Where this happened, the ratio of TTC to FS was substantially increased, and this may be an factor which should be borne in mind when assessing the effectiveness of disinfection in individual samples.

It is not possible at present to adequately characterise the influent sewage in terms of pathogenic organisms.

(c) Toxicity

The toxicity of peak flow influent sewage to oyster embryos was very constant. EC_{50} values lay between 1.18 and 1.90 (% sewage). The toxicity of a single low flow sample was less, with an EC_{50} of 6.96%. Microtox was uniformly less sensitive, but showed a wider range of variation in response to the three high flow sewage samples. Both tests agreed that the single low flow sample was least toxic.

(d) Relationships between variables

Routine chemical variables, Cu, Zn, Fe and Al all displayed a similar pattern of variation in 17/18 July samples. Variable peaks were more closely related to time than to bacterial load, although this conclusion is subject to the assumption that isolated peaks in TTC numbers were reliably observed.

A comparison of TTC numbers with Cd, Pb, Cr, $CHCl_3$ and phenol concentrations on 17/18 July could only be made for two samples, and it would not therefore be safe to infer any trend. It is clear, however, that there is not a simple relationship between concentrations of these variable and bacterial load; marked differences in metal concentrations between samples were not matched by equivalent differences in bacterial load.

5.6.2 Effects of disinfection on sewage chemistry and toxicity

(a) Routine chemistry

Although average values suggest a repeated but not significant increase in BOD and COD values following the addition of PAA to sewage, this obscures a consistent pattern in which concentrations of these variables was higher in disinfected sewage than in undisinfected sewage during periods of low flow; the converse was generally the case during periods of high flow.

Values of pH were consistently lower in treated than in untreated sewage by 0.2-0.4 units.

b) Inorganic determinands

Neither screening nor the addition of PAA affected the concentrations of the majority of inorganic determinands in sewage samples. In general, post-disinfection concentrations were close to pre-disinfection concentrations. The most notable exception was Pb, levels of which were consistently higher in samples from point 2; it must be noted, however, that these observations are based on a limited number of samples. There was a tendency for heavy metal concentrations to be higher in point 2 samples than in point 1 samples on 11/12 September.

Effects of disinfection on partitioning - Baseline trial

Concentrations of dissolved metals ($\mu g\ l^{-1}$) as a proportion of total (dissolved plus particulate) in point 1 and point 2 sewage samples were plotted for Al,

Fe, Cu and Zn as bar charts to permit a broad comparison of trends in partitioning prior to disinfection (Figure 5.3). In the majority of instances, a higher proportion of the metals was present in the dissolved phase in pre-screen samples than in post-screen samples. This trend was more marked for Al, Fe and Cu than for Zn; in one sample, dissolved Zn was present in higher proportion after the screen than before. Overall, where differences occurred, these indicated that (perhaps as a result of contact with solids collected on the screen) the screening process tended to reduce the relative amount of dissolved metals.

Effects of disinfection on partitioning - Fixed dose trials (14/15 August and 11/12 September)

In contrast to the above observations, there was a consistent trend for the fraction of Al, Fe, Cu and Zn present in the dissolved phase to be higher in samples from point 2 than from point 1, suggesting that the disinfection process might favour the solubilisation of these metals (Figures 5.4 and 5.5). Some exceptions to the trend were notable; in particular, one 0600 h sample from the 14/15 August trial in which the proportion of metals associated with the particulate phase was appreciably higher in the post-contact-tank sample.

(c) Residual oxidants

The 'total bromine' values were obtained by direct oxidation of DPD and subsequent uv measurement. Such a measurement would include any chlorine, bromine and bromamines that are present. However, the presence of chlorine was considered unlikely because PAA does not oxidise chloride to any significant extent.

Bromine and bromamines are likely to have been present because PAA is capable of oxidising bromide to bromine ('Identification of reaction products and assessment of toxicity and mutagenicity', progress report to the NRA, November 1990); any free bromine formed as a result of this reaction is likely to have been converted to bromamine due to the presence of ammonia. In addition, iodine could also be present as a result of the oxidation of any iodide by PAA and would be included in the 'total bromine' analysis.

Figure 5.3 Concentrations of dissolved Al, Fe, Cu and Zn as proportions of total concentrations (dissolved plus particulate) in point 1 and point 2 sewage samples taken on 17 - 18 July 1990

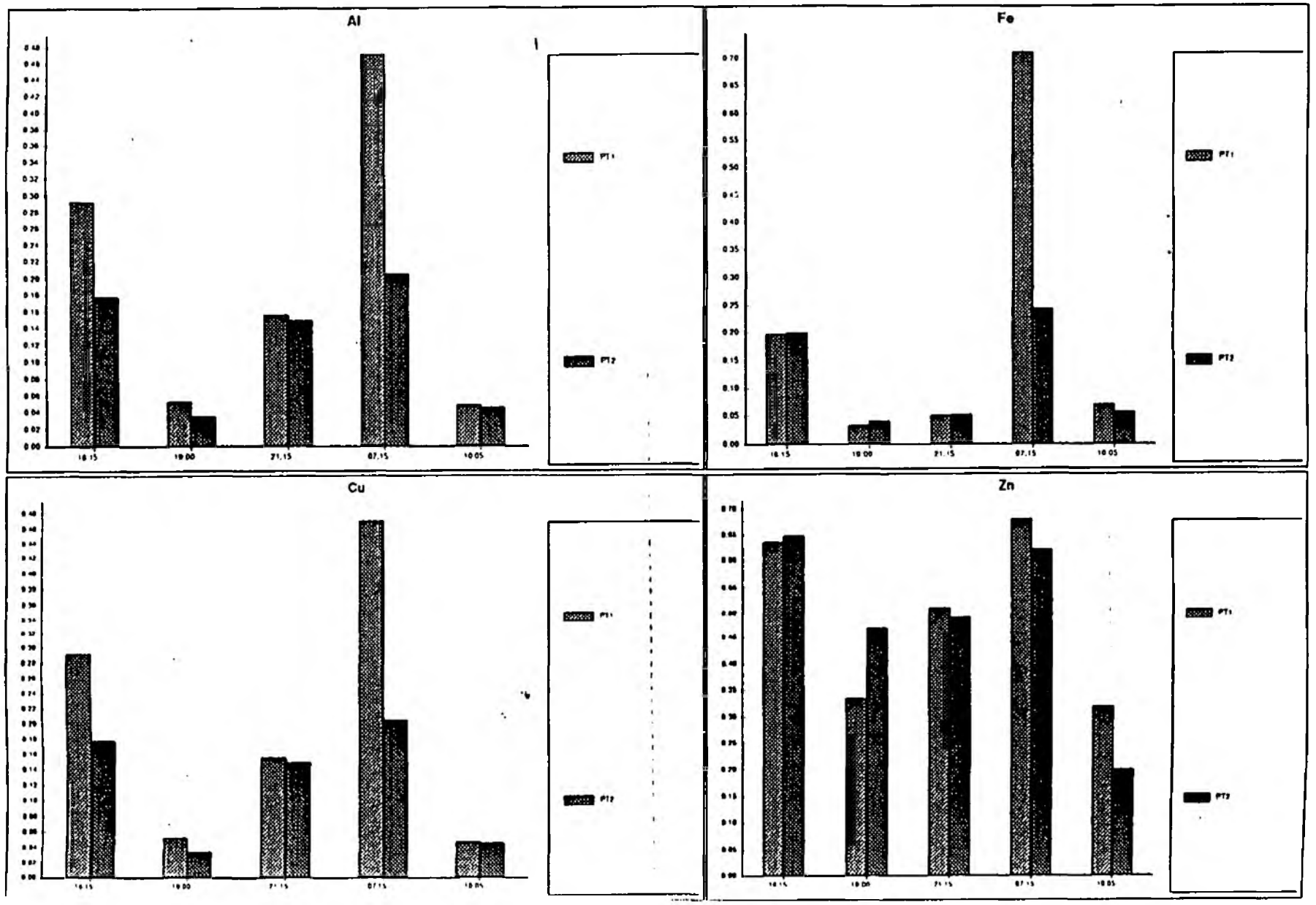


Figure 5.4a Concentrations of dissolved Al, Fe, Cu and Zn as proportions of total concentrations (dissolved plus particulate) in point 1 and point 2 sewage samples taken on 14 - 15 August 1990

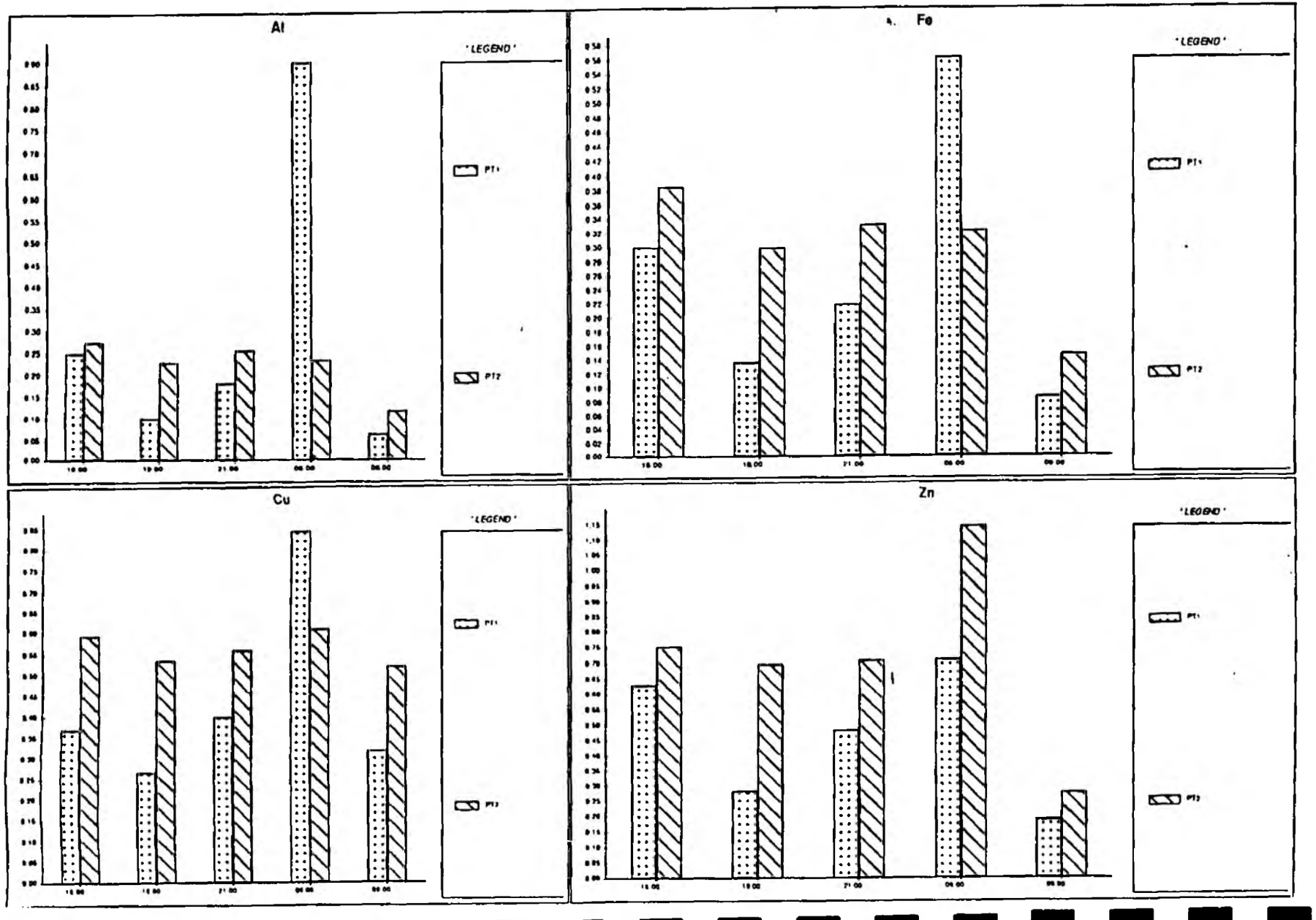


Figure 5.4b Concentrations of dissolved Pb, Cd, Ni and Cr as proportions of total concentrations (dissolved plus particulate) in point 1 and point 2 sewage samples taken on 14 - 15 August 1990

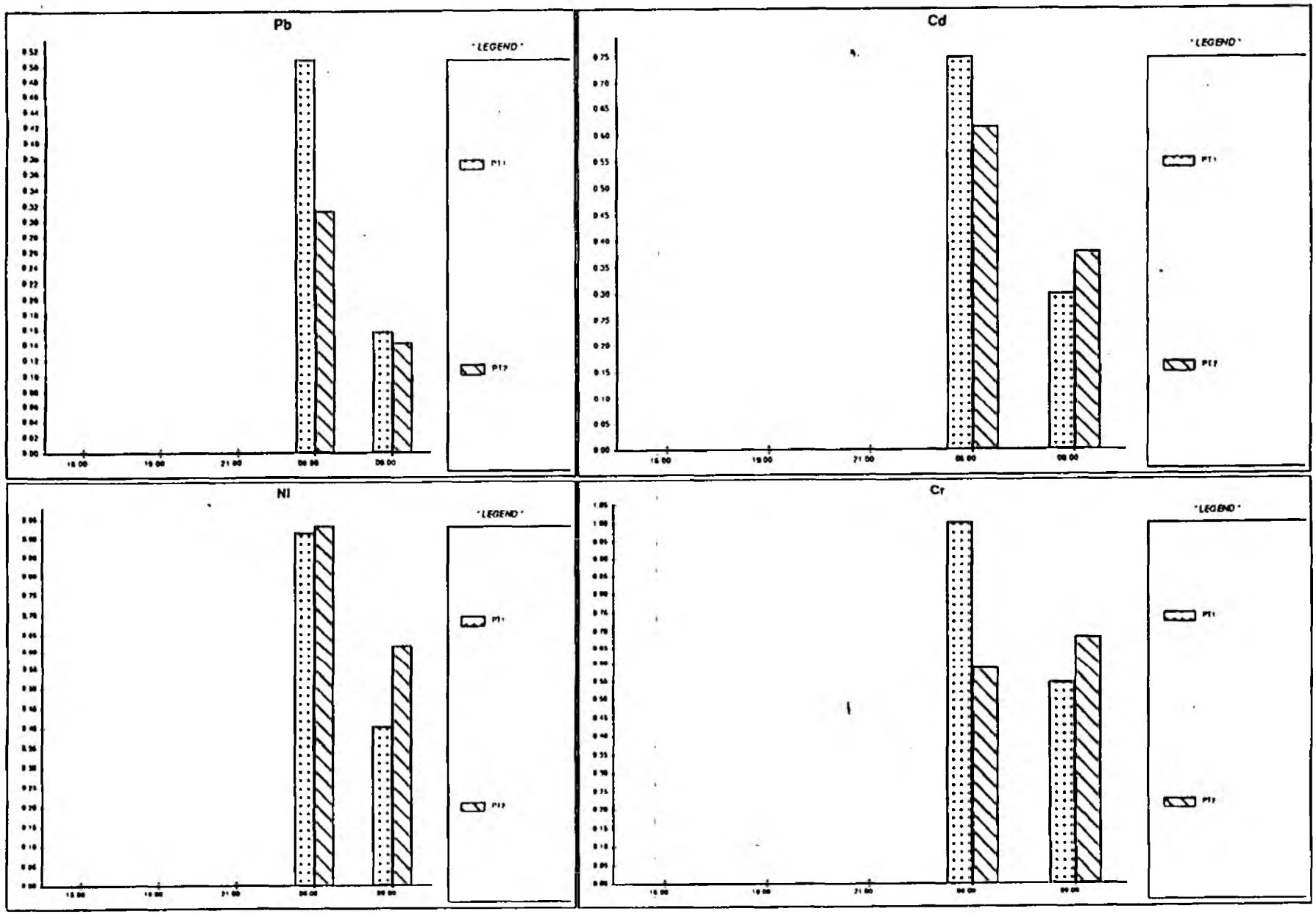


Figure 5.5a Concentrations of dissolved Al, Fe, Cu and Zn as proportions of total concentrations (dissolved plus particulate) in point 1 and point 2 sewage samples taken on 11 - 12 September 199

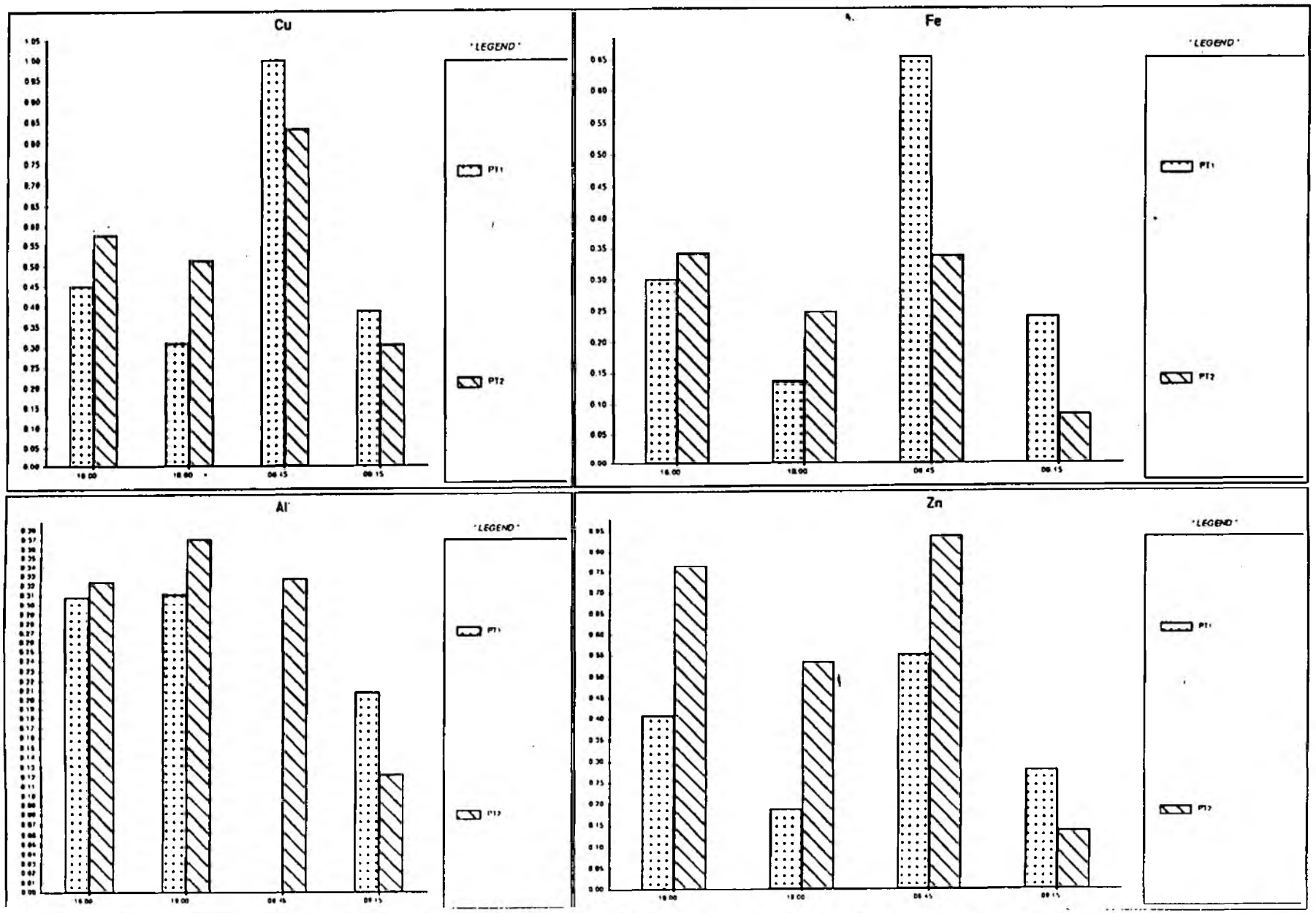
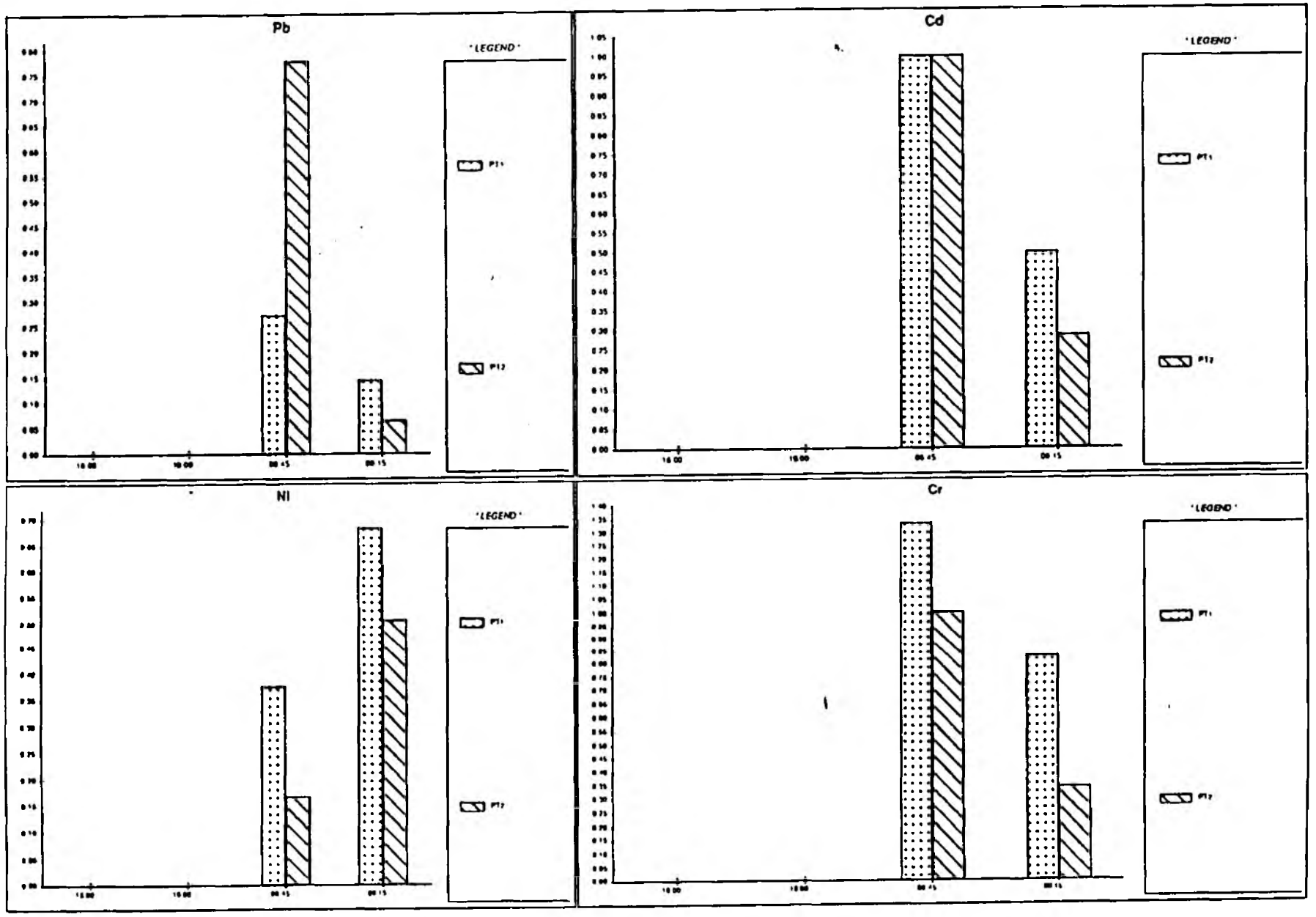


Figure 5.5b Concentrations of dissolved Pb, Cd, Ni and Cr as proportions of total concentrations (dissolved plus particulate) in point 1 and point 2 sewage samples taken on 11 - 12 September 1990



MCA, which would interfere in the PAA measurement, is unlikely to have been present in the sewage at measurable levels, since PAA does not oxidise chloride to chlorine to any significant extent, nor was it detected prior to addition of PAA. Therefore, there should have been little interference in the PAA measurements. Similarly, DCA is unlikely to have been present because of the lack of reaction of PAA with chloride. Anything detected in these analyses (addition of KI and allowing to react for 2 minutes before taking UV absorbance reading) is more likely to be due to organic bromamine compounds (produced as a result of the oxidation of bromide by PAA and subsequent reactions of bromine and/or bromamine with organic substances in the sewage) which are capable of oxidising KI in a similar way as DCA; consequently these measurements were expressed as 'organic bromamines' in Tables 5.3 and 5.4. As shown in Tables 5.3 and 5.4, PAA, H_2O_2, 'total bromine' and 'organic bromamines' were all below detection limit in samples taken prior to the contact tank (sampling site 1). The highest concentrations of residual disinfectants (PAA and H_2O_2) and by-products ('total bromine' and 'organic bromamines') were found immediately after the contact tank (sampling site 2) at periods of low loading (e.g. 0600 hours, Table 5.3 and 0645 hours, Table 5.4) and decreased with distance away from the sewage works (sampling site 4a - discharge to sea and 4b - edge of sewage plume in the sea), although they were still present at the point of discharge to sea. Residual H_2O_2 concentrations were always higher than PAA, indicating that the latter was consumed more rapidly, although some of the difference may have been due to a delay in analysing PAA which required centrifugation. At periods of high loading (e.g. 0900 hours, Table 5.3 and 0915 hours, Table 5.4) most or all of the disinfectant was consumed in the contact tank.

(d) Complex organics

Similar types of compound were identified in disinfected and undisinfected samples, and the compounds identified were typical of those normally found in sewage samples.

As has been noted above, the results of GCMS analysis can at best be interpreted at an order-of-magnitude level of certainty. It was observed that disinfected sewage samples were less complex than undisinfected sewage samples,

Table 5.3 - Residual disinfectants (mg l^{-1}) measured in sewage and seawater samples collected at Trevaunance Cove STW and discharge plume on 15 August 1990

Sampling time	Site	Bromine as Br_2	Hydrogen peroxide	PAA	Organic bromamine as Br_2
0600	1	<0.02	<0.01*	<0.01	<0.02
	2	0.11	12.3	2.0	0.45
	4a	0.07	0.4	0.06	0.05
	4b	0.04	<0.1	<0.01	0.11
0900	1	<0.02	<0.01*	<0.01	<0.02
	2	0.10	3.1	0.02	<0.02
	4a	0.03	<0.01	<0.01	<0.02
	4b	0.03	<0.1	<0.01	<0.02

* measured in undiluted sewage

Table 5.4 - Residual disinfectants (mg l^{-1}) measured in disinfected (Site 2) and undisinfected (Site 1) Trevaunance Cove sewage and receiving waters (4a, 4b) collected on 11/12 September 1990

Sampling time	Site	Bromine as Br_2	Hydrogen peroxide	PAA	Organic bromamine as Br_2
11.9.90					
1600	1	n/t	n/t	n/t	n/t
	2	0.35	9.9	<0.01	<0.02
	4a	0.12	0.5	<0.01	<0.02
	4b	n/t	n/t	n/t	n/t
1900	1	n/t	n/t	n/t	n/t
	2	0.12	2.8	<0.01	0.16
	4a	0.10	0.5	<0.01	0.14
	4b	n/t	n/t	n/t	n/t
12.9.90					
0645	1	<0.02	<0.1	<0.01	<0.02
	2	0.06	16.3	0.84	0.45
	4a	0.09	1.7	0.12	0.05
	4b	0.04	<0.1	<0.01	<0.02
0915	1	<0.02	<0.1	<0.01	<0.02
	2	0.09	<0.1	<0.01	<0.02
	4a	<0.02	<0.1	<0.01	<0.02
	4b	0.05	<0.1	<0.01	<0.02

but that this comparison is confounded by the difference in collection date between the samples and the possibility that gross differences in influent sewage characteristics may exist between sampling times.

Bearing this in mind, the total concentrations of complex organic compounds (basic and acidic fractions) were calculated for all three sampling dates (Table 5.5). It is clear that the range of variation is relatively greater in sewage samples than in seawater samples, and that there is no obvious relationship between the application of disinfectant and total concentration. The range of concentrations observed was, however, small considering the order-of-magnitude precision of the analysis.

Table 5.5 - Approximate total organic chemical concentrations (mg l^{-1}) in samples of sewage and seawater collected at Trevaunance Cove STW on three dates

Date	Acidic fraction 2	Acidic fraction 4a	Basic fraction 2	Basic fraction 4a
17.7	25	3	30	1.5 (undisinfected)
5.9	5	2	1.3	0.6 (20 mg PAA/l)
11.9	19.8	0.9	7.8	1.1 (20 mg PAA/l)

(e) Phenols and Organohalogen compounds

Phenolic compounds (predominantly phenol) displayed a marked diurnal variation in concentration, but were not either consistently or (probably) significantly elevated in point 2 samples compared to point 1 samples.

VOX compounds were represented mainly by chloroform, which was present in similar concentrations in disinfected and undisinfected sewage, and at similar levels during all three sampling periods. Other determinands were present in detectable quantities mainly in the morning samples of each sampling period. There was little evidence of any clear or pronounced diurnal variation in concentrations.

(f) Toxicity

Disinfected sewage was of similar, or lower, toxicity to both microtox and oyster embryos than was undisinfected sewage. The addition of PAA did not therefore, under the conditions of these trials, increase the toxicity of sewage samples. The EC_{50}s measured for undisinfected sewage are typical of values obtained in other studies for domestic effluent.

5.6.3 Effects of sewage chemistry on disinfection efficiency

The data sets obtained from trials conducted on 14/15 August (20 mg l^{-1} PAA) and 11/12 September (20 mg l^{-1} PAA) were examined to establish whether the effectiveness of disinfection could be related to identifiable sewage characteristics. A degree of caution should be observed in inferring relationships, for two reasons. Firstly, there is an unquantified but real level of uncertainty attached to each analytical value; this uncertainty is largest in respect of the organic chemical determinations. Secondly, a full comparison of values can be made for only three to five observations on each of the above occasions.

Phosphate and pH were not systematically related to bacterial kill in any of the trials. BOD, COD, suspended solids and ammonia concentrations were monotonically related to bacterial kill, low values of the former being associated with more effective disinfection.

Fe concentrations were monotonically related to kill efficiency in 11/12 September samples, but these variables were less clearly related in 14/15 August samples. Concentrations of other metals displayed an inconsistent relationship with bacterial kill rate, with a relatively wide range of metals levels being observed at lower levels of disinfection efficiency. Cd, Pb, Cr and Ni concentrations appeared to be inversely related to bacterial kill rate, but these data are based on only two observations; a comparison with data for metals for which more observations are available suggests that this apparent relationship is a result of aliasing.

The chemical variable most consistently related to disinfection efficiency on 14/15 August was chloroform; a monotonic decline in log bacterial kill was observed with increasing chloroform concentration. The level and range of chloroform concentrations was too low, however, to attribute great significance to this relationship.

VOX and phenol concentrations in 11/12 September samples were inversely monotonically related to bacterial kill rate; in contrast, kill rate increased with increasing hydrogen peroxide concentrations. This latter observation suggests that good bacterial kill is achieved when an excess of disinfectant is available. The former observation suggests the possibility that organic compounds may exert a demand on PAA which reduces its capacity to kill microorganisms.

Toxicity (Microtox, too few relevant observations to use oyster test in comparisons) in 14/15 August samples was also monotonically related to bacterial kill rate, but did not bear a similar relationship to other measured variables. There appeared to be an inverse relationship between bacterial kill rate and toxicity to both Microtox and oyster embryos in 11/12 September samples, but this observation is based on a comparison for only two samples. Toxicity was greater when the concentration of heavy metals and, particularly, organic chemicals, was higher in influent sewage. The relationship between toxicity and organic chemicals (primarily phenol and chloroform), when considered together with the apparent reduction in complex organics post-disinfection revealed by GCMS analysis, suggests the possibility that toxicity may be useful as a surrogate measure of organic chemical loading.

5.6.4 Chemistry and toxicity of receiving water samples

(a) Residual oxidants

As noted above, H_2O_2 and PAA were observed in samples taken at the point of discharge (4a) during periods of low loading on both occasions (14/15 August and 11/12 September.

Where measured, PAA and H_2O_2 concentrations were below detection limit at the edge of the plume (site 4b), whereas low levels of 'total bromine' and, in one case, 'organic bromamines' were still found at the edge of the sewage plume.

(b) Complex organics

On reviewing the compounds listed for seawater, it is not possible to say with certainty whether any of these compounds were present as a result of PAA treatment. There is a strong possibility that the data reflect gross differences in the contents of the sewage between times.

(c) Phenols and organochlorine compounds

Phenol was present at low levels at the point of discharge on 17/18 July and 14/15 August, but reached very much higher concentrations in samples collected at the same point on 11/12 September. AOX values on 11/12 September were on occasion higher in seawater samples than in corresponding sewage samples, but measurements were not made on earlier dates and a temporal comparison could not be made.

Phenols and chlorinated phenols appear to have been unaffected by PAA treatment. PAA could not have produced these compounds (because PAA does not oxidise chloride to form chlorine, and consequently does not produce organochlorine compounds).

It is interesting to observe that phenol does not appear to have been oxidised by PAA, i.e. little difference in phenol concentrations before and after disinfection (where both results are available), although caution is needed when interpreting these results, as there could be difficulties with phenol analyses in the presence of high concentrations of other organic (humic) substances.

VOXs were unaffected, i.e. neither produced nor destroyed by PAA. The production of haloforms (normally produced from chlorination) other than bromoform would not be expected (because PAA does not oxidise chloride). The production of bromoform might have been expected (as a result of oxidation of

bromide and subsequent production of organobromine compounds). However, laboratory experiments referred to earlier (Crathorne *et al* 1991) which involved PAA treatment of humic acids and bromide, showed no bromoform production, although organobromine compounds (measured as AOX) were produced.

There seems to have been a substantial increase in AOX as a result of PAA treatment (but only two results have been obtained). This is likely to have been due to production of organobromine compounds (from the reaction of PAA with bromide in the sewage), in accordance with results of experiments referred to above. However, the increase in AOX at the point of discharge (compared with the contact tank) in the 1600 hour and 0915 hour samples is difficult to explain but may be a result of increased contact time in the effluent pipe. Interference from other compounds in the seawater (inorganic halide?) could be a problem, or the presence of particulate matter which could result in inaccurate AOX measurements. Further work would be needed to develop separate analyses of the liquid and particulate portions of the samples.

(d) Inorganics

Dissolved oxygen concentrations in receiving water samples were always in excess of 90%, and salinity measurements indicated that in most cases the samples were well-diluted at the time of sampling. BOD, suspended solids and NH_3 levels were substantially reduced at the distance of point 4b from the point of discharge.

A comparison was made between the levels of phosphate in pre- and post-disinfection samples (points 1 and 2 respectively) and in samples collected from the centre (point 4a) and edge of the discharge plume (point 4b). Concentrations of phosphate in samples from the latter two points were considered likely to provide an indication of the degree of dilution which the sewage had undergone at the precise point and time of sampling. In order to distinguish actual dilution from the contribution of background seawater phosphate levels, the ratio between concentrations in samples at points 2, 4a, and 4b was examined.

Concentrations in sewage

Concentrations were closely similar at points 1 and 2 during the dose-setting trials (2a, 2b, 2c). There was a trend for concentrations to decline with time during the 24 h sampling periods on 31 July and 1 August, but no such marked trend on 2 August. Phosphate levels at points 1 and 2 were also similar during the 20 mg l^{-1} PAA trial on 14/15 August (run 3), but showed no clear diurnal pattern and remained at or around a concentration of 10 mg l^{-1}. During the final 20 mg l^{-1} PAA trial on 11/12 September, there was a clear tendency for phosphate levels at point 1 to exceed those at point 2.

Concentrations in discharge plume

Phosphate levels in samples at point 4b varied relatively little over the entire sample series, suggesting that these values represent the approximate background seawater concentrations. On occasions when concentrations at point 4a were lowest, the difference between 4a and 4b was relatively small; this is unlikely to be due to a lower dilution at these times, for reasons which become apparent when the relationship between concentrations in the sewage and at point 4a is considered.

In order to determine whether levels of phosphate at point 4a reflected more strongly the rate of dilution or the actual levels in the discharged sewage, values for points 2, 4a and the ratio of these (4a/2) were considered. The ratio expresses the effective rate of dilution at the point of sampling. Over the five sets of samples for which field data were available, there was no consistent pattern of relationship between concentrations at point 2 and concentrations at point 4a. In fact, positive, negative, and random relationships were observed. The relationship between point 4a levels and the ratio of 4a/2 levels was much more consistent, with a positive relationship observed for each data set. This relationship was maintained when all the relevant data were combined and a regression analysis performed on the log-transformed values. The overall correlation coefficient was 0.906, and the regression equation was:

$$\text{Log } 4a = 0.615 + 0.854 \log 4a/2$$

This appears to indicate that concentrations at point 4a are highly dependent on the degree of dilution, but only weakly or inconsistently dependent on the actual levels or amounts of phosphate in the sewage being discharged. Consequently, the levels of phosphate in samples from point 4a can be regarded as a reasonable indicator of the fraction of a given flow of sewage represented by the sample, but not of the strength or chemical composition of the sewage.

Linear regression of the untransformed data gave an intercept value of 0.022, and a slope of 6.08, suggesting that on average the background seawater concentrations of phosphate might be of the order of 22 $\mu g\ l^{-1}$ and that dividing the 4a phosphate levels by 6 would provide an estimate of the proportion by which the sewage has been diluted at the point of sampling. Actual concentrations at point 4b were as low as 8 $\mu g\ l^{-1}$, with a median value of 25 $\mu g\ l^{-1}$.

It must be emphasised that this treatment of the data should be regarded as purely a guide to interpretation, and not a definitive analysis; in particular, extrapolation beyond the limits of the values recorded would yield the prediction that sewage contains 6.1 mg phosphate per litre. A more realistic analysis would require the assumption of an asymptotic relationship; the region of such a curve within which the present observations lay would be essentially linear, however and would yield a relationship similar to that reported here.

Heavy metal concentrations were low in receiving water samples, although Zn values appeared to be elevated in samples collected at points 4a and 4b on 11/12 September.

(e) Toxicity

A marked contrast was apparent between the toxicity of water samples collected on 14/15 August and those collected on 11/12 September. The latter samples proved to be acutely toxic to oyster embryos. Relatively high concentrations of phenol were associated with these samples, but it is not possible to determine whether or not the observed toxicity was directly associated with the discharge of disinfected sewage. Concurrent measurements of salinity indicated, however, that up to 10% of the sample by volume was of non-saline origin.

5.7 Conclusions

5.7.1 Microbiological efficacy of PAA

PAA treatment of domestic sewage did not succeed in achieving the required reduction in faecal bacterial numbers, and did not appear (on the basis of presently-limited data) to be effective in reducing the numbers of pathogenic bacteria and viruses. A review of the results of the analysis for 'non-routine' microorganisms concluded that the data acquired in this study could only effectively be used to indicate presence or absence.

The efficiency of the treatment process may have been impaired by the design of the disinfectant contact tank; a tracer study indicated that much of the influent sewage would have a contact period of two minutes, rather than the five minutes recommended for effective bacterial kill. However, even this limited contact time was sufficient to allow the elimination of most of the disinfectant.

PAA treatment did not achieve an adequate reduction in numbers of faecal bacteria in mussels sampled from the vicinity of the discharge, or in water samples collected nearby. Very little effect was observed on numbers of F^+ coliphage associated with these mussels.

5.7.2 Environmental effects of disinfection with PAA

PAA treatment resulted in small increases in the concentration of oxidants in the effluent sewage. There was some evidence that BOD, COD and heavy metals levels might be elevated in effluent sewage at some times, but this is open to some question. In general, disinfection led to an increase in the proportion of total heavy metals present in the dissolved phase. Organic contaminants were, overall, neither clearly destroyed nor created by the disinfection process.

The addition of PAA to the sewage, and the consequent chemical changes, did not increase observed toxicity.

Very high toxicity was observed in water samples collected in the discharge plume on 11/12 September, associated with exceptionally high concentrations of phenol. This may have been a consequence of an impairment of water quality by other inputs to the area. The observed toxicity was higher than could be accounted for by the presence of sewage in the samples.

The relatively high concentration of 'organic bromamine' (0.11 mg l^{-1}) in one discharge plume sample suggests that relatively stable organic bromamines might be present. However, it is difficult to explain why the concentration should be higher at the edge of the plume (point 4b), compared with the point of discharge (4a), and in the absence of any other, similar results, no conclusions can be drawn from this single observation. It must be stressed that the 'organic bromamine' measurement is an indirect measurement only. The presence of organic bromamines is based on i) the assumption that some organic bromamines behave similarly to dichloramine/organic chloramine in the DPD photometric technique for the analysis of chlorine residuals, and ii) the observation that organobromine compounds are more likely to be formed (from PAA and bromide reacting with organic substances) than dichloramine/organic chloramine. Confirmation of the presence of organic bromamines would require the development of novel, sophisticated techniques involving extraction, derivatisation and identification by GC-MS.

6. TRIALS WITH PERACETIC ACID AND SECONDARY EFFLUENT

6.1 Introduction

This chapter reviews the disinfection trials using PAA with a secondary effluent from Porthtowan STW. Disinfection trials using PAA on a fully treated sewage effluent were carried out at Menagwins STW, in the SWW region, during 1989/1990 (Vincent and Realey 1990). Several problems arose as a result of dosing the peracetic acid prior to the humus tanks, which provided a contact time for the PAA to kill off micro-organisms. The problems included:

(i) an increase in the BOD, suspended solids and ammoniacal-N present in the final effluent;

(ii) a reduction in the pH of the final effluent;

(iii) the increased growth of filamentous-chain bacteria ('sewage fungus') in the effluent channel of the sewage works and in the receiving water;

(iv) a significant level of hydrogen peroxide present in the sewage works effluent.

Therefore there was a need to study the disinfection of secondary effluent using PAA with a separate contact tank. Porthtowan STW produces a suitable sewage effluent quality and the hydraulics of the system allowed for the construction of a contact tank post humus tanks. Also the environmental impact could be studied as the discharge was to a Class 2 river, which became Class 3 approximately 180 m below the discharge point, away from any amenity use. The aim was that trials at Porthtowan would clearly answer all the questions raised by the trials carried out at Menagwins STW.

NRA interim report R&D 231/1/SW (Roddie *et al* 1991) describes in detail the methods and results of this trial. This chapter summarises the work and discusses the findings.

6.2 PAA dosing at Porthtowan

Figure 6.1 shows the layout of Porthtowan STW. The site is an inland treatment works which discharges fully treated effluent to a small river, that in turn flows into the sea at Porthtowan beach, approximately 2 km downstream. The works serves an estimated resident population of 1700, which gives an average dry weather flow of 302 m^3 day-1. This rises in the summer to 520 m^3 day-1 due to a population increase of about 1200. The source is mainly residential although there is also a moderate level of infiltration. In the summer local caravan sites are the main source of the increase in influent.

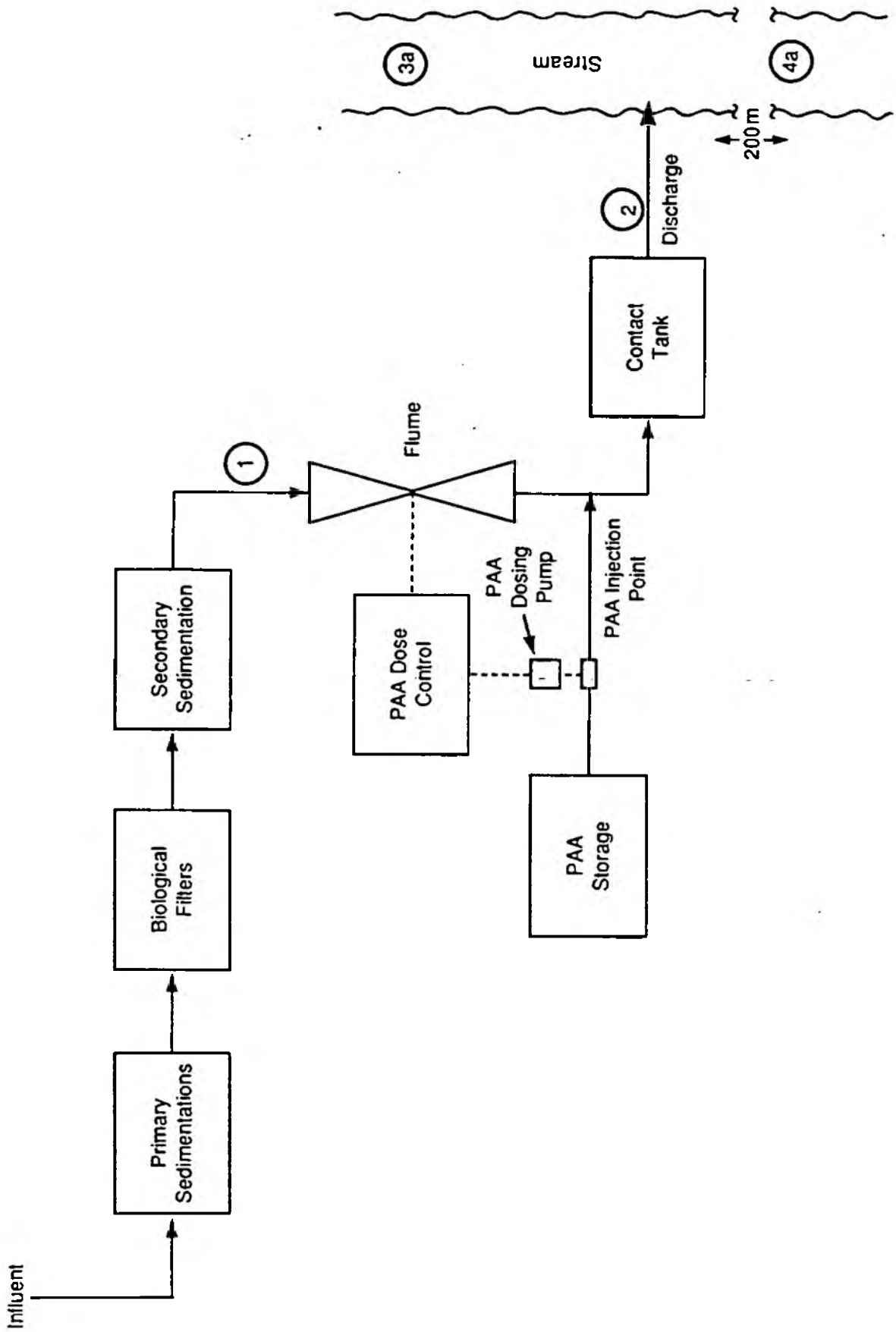
The sewerage system at Porthtowan STW has no overflows, the only one that exists is at the pumping station from Porthtowan situated 1 km downstream of the works. Therefore all flows to the sewers pass to the works for treatment, and they vary widely from a DWF of 3.5 litres/second up to greater than 30 litres/second.

Sewage passes from the inlet to three primary sedimentation tanks and then on to three biological filters followed by three secondary sedimentation tanks before discharge to the river via a common effluent channel. These trials used a contact tank built prior to the discharge of effluent to the river, to ensure a sufficient retention time for the PAA to have effect. Interox recommended a tank with a retention time of between 6-9 minutes for effective kill. The tank was a cylinder with a diameter of 2 metres and height of 2 metres, which gave a volume of 6.28 m^3 and the desired contact time. There were three internal baffles to ensure proper mixing with no short circuiting of flow.

Directly prior to the contact tank a short flume section was installed above which an ultrasonic level-probe was situated to give an accurate measure of the effluent flow. Due to slight operational problems the effluent flow varied considerably, in relation to the influent, rather than demonstrating a smoothed out diurnal flow variation.

A permanent chart record of effluent flow was obtained from the ultrasonic level used to determine the PAA dosing level. In addition to this flow rate the time of sampling at point 2 (Figure 6.1) was measured directly by finding the time taken for a ten litre bucket to fill, this being averaged over three tests.

Figure 6.1 Flow diagram of Porthtowan Sewage Treatment Works with PAA dosing



An estimate of river flow was obtained just above point 4b (Figure 6.2), so that the impact of the sewage works discharge could be determined. This was done by means of determining the time taken for floats to travel a distance of 50 m over a suitable reach and measuring the width and depth of the river, from these the river flow was calculated. This flow was then adjusted by means of catchment area to produce an estimate of the river flow at a point 200 m downstream of the discharge from Porthtowan STW.

The PAA was dosed through a sparge to the effluent channel directly before the contact tank, after the flume section (Figure 6.1). Dosing was based on flow-proportional control. A 4 to 20 milliamp control signal, from the ultrasonic level probe before the flume section, drove the dosing pump inverte. The pump maintained a predetermined dose level such that the dose was flow proportional.

6.3 Study design and sampling sites

The study was conducted in three phases:

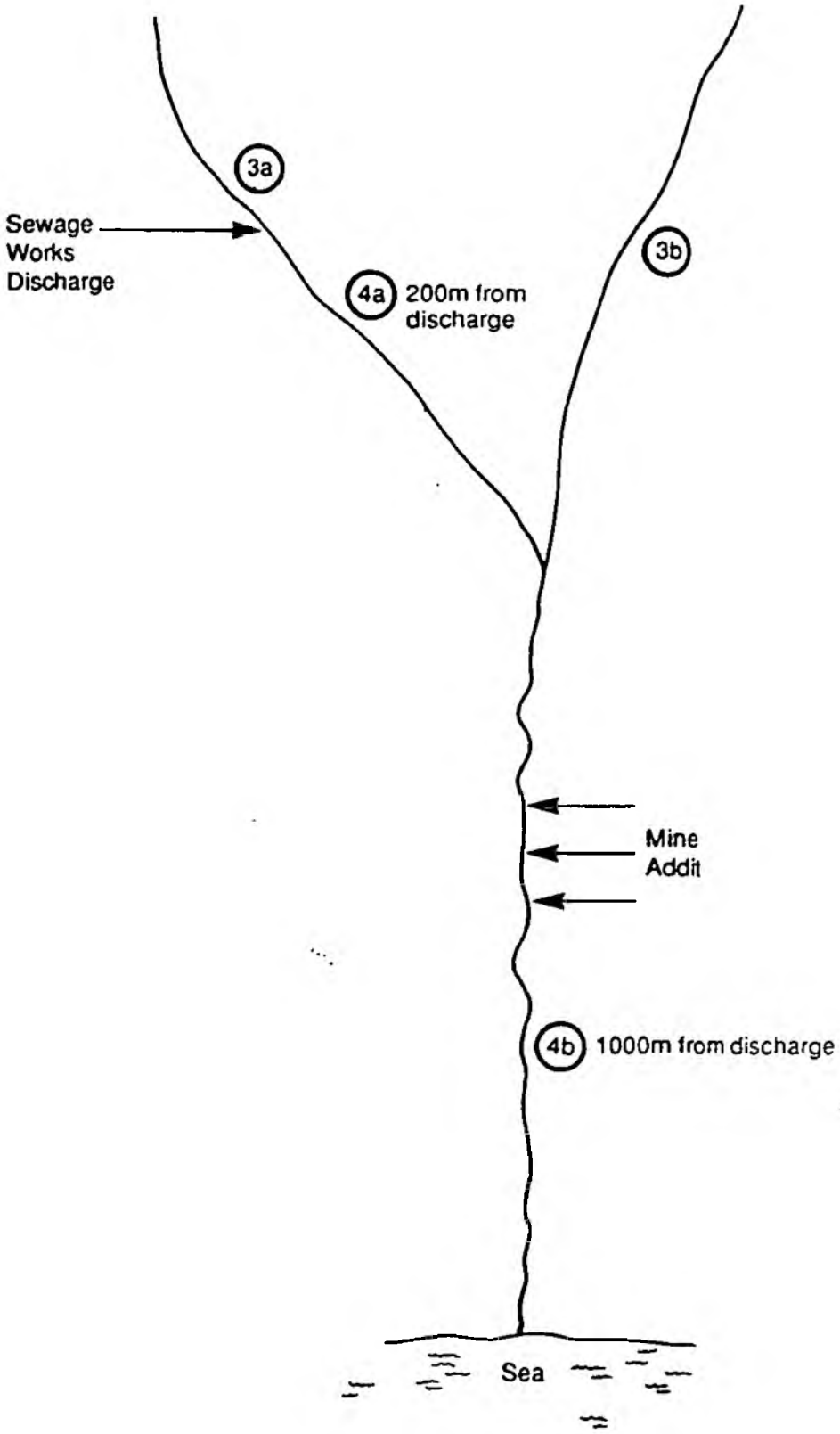
1) A **baseline survey** (30 October 1990, 22 h trial) to establish background information on the chemistry of the treated sewage prior to the commencement of the disinfection process.

2) **Dose-setting trials** (27 and 28 November 1990, 8 h trials) to establish an appropriate dose level for subsequent trials. Doses of 6 and 4 mg l^{-1} PAA were assessed respectively. These trials assessed the effects of extended contact time.

3) **Disinfection evaluation trials** (4-5 December and 11-12 December 1990, 24 h trials) conducted at the more effective of the two dose rates evaluated during Phase 2.

In addition, the retention time of the contact tank was investigated over two 30-min periods on 19 November 1990. Lithium salt was added (stock solution concentrations of 639 and 656 mg l^{-1} respectively) to the sewage prior to the contact tank, and samples collected at one-minute intervals at the outflow from the tank.

Figure 6.2 PAA Sampling points in the stream at Porthtowan



A total of six sampling sites were used (Figures 6.1 and 6.2). Sample points 1 and 2 were located before and after the contact tank respectively. Sample point 3a was in the receiving stream just above the point of discharge and sample point 4a was located in the receiving stream approximately 300 m downstream of the point of discharge. In order to place the in-stream measurements in a local context, additional sample points were located in a nearby stream above its confluence with the Porthtowan river (Site 3b) and in the Porthtowan river below the confluence (Site 4b).

All samples were synoptic; subsamples for each set of determinands were withdrawn from a single, bulk sample collected at each time. Sample point 1 was sampled using a 5 litre plastic bucket, and point 2 with a 10 litre plastic bucket. Five-litre buckets were used to sample all river points. It was not practicable to collect samples from each point simultaneously, nor to lag sample timing in order to follow the flow through the treatment works; the distance between sample points required some 45 minutes to cover during sample collection. Consequently, whilst measurements on a single sample are directly comparable, some caution must be observed when comparing measurements between samples.

6.4 Parameters investigated and analysis

Chapter 3 describes the chemical and microbiological determinands studied and and analytical methods used. Table 6.1 shows which tests were used on which medium. Toxicity was assessed using both the oyster embryo-larval test and Microtox, as described in Chapter 5 on the Trevaunance work. In addition, *in situ* effects in the stream were investigated by measuring changes in feeding rate in *Gammarus* deployed in cages at sites above and below the point of discharge.

Samples were collected at the points and with the frequency indicated in Tables 6.2 to 6.4 for each group of determinands. Where 12 or more samples are indicated, collection was made at regular intervals (from 1 h to 2 h). Where five samples are indicated, these were collected, typically, at 16.00, 18.00, 20.00, 06.00 and 08.00 hours during a trial conducted between 10.00 and 10.00.

Where two samples are indicated, these were taken at nominal peak and low flows at 06.00 and 09.00 hours during the second half of the trial.

Table 6.1 - Chemical and microbiological measurements undertaken during Porthtowan disinfection trials

Group	Medium	Variable
Routine chemistry (RC)	Sewage	BOD,COD,NH3,PO4,SS,TON,pH,Temp
	Water	As above, plus DO
Routine microbiology (RM)	Sewage	TTC, FS
	Water	TTC, FS
Non-routine Microbiology (NRM) Staphylococcus	Sewage	Enterovirus, rotavirus, F+ coliphage, Salmonella, Campylobacter, Pseudomonas,
Trace metals (TM)	Sewage	Cr,Cd,Pb,Ni,Cu,Zn
	Water	Cr,Cd,Pb,Ni,Cu,Zn
Volatile organohalides (VOX)	Sewage	$CHCl_3$, $111C_2H_3Cl_3$, CCl_4, C_2HCl_3,
	Water	$CHCl_2Br$, C_2Cl_4, $CHClBr_2$, $CHBr_2$
Adsorbable organohalides (AOX)	Sewage	'Total' AOX
	Water	
Complex organics	Sewage	GCMS broad spectrum scan
	Water	
Phenol and Chlorinated phenol (P/CP)	Sewage	Phenol, 2-, 4-, di-, tri, penta-
	Water	chlorophenol
Residual oxidants (RO)	Sewage	PAA, H_2O_2, 'bromamine', 'organic
	Water	bromamine'

6.5 *In situ* toxicity assessment

The feeding rate of the amphipod *Gammarus pulex* L. has been used to indicate the presence of water pollution at several running water sites (Maltby *et al* 1990a, Crane and Maltby 1991). *G. pulex* is an important detritivore in many river systems (Kaushik and Hynes 1971) and the feeding rate of this animal is depressed by a range of toxic compounds (Naylor *et al* 1989). Methods have been developed by WRc and Sheffield University for the use of *G. pulex* feeding rate in standard laboratory water column or sediment tests (Maltby and Naylor 1990, Roddie *et al* 1990) and as an *in situ* water quality monitor (Maltby *et al* 1990a, Crane and Maltby 1991). Toxicity tests in fixed or mobile laboratories have the advantage of greater experimental control and allow the test operator to randomise treatments. *In situ* tests have the advantage of greater relevance to the system under investigation, because they are placed within the system itself. However, there are some serious methodological problems common to many *in situ* tests.

Table 6.2. Sampling requirements for Run 1, 30 October 1990 (number of samples at each site)

Determinand	Site 1	2	3a	3b	4a	4b
RC	12	12	12	12	12	12
RM	0	0	0	0	0	0
NRM	0	0	0	0	0	0
TM (total)	5	5	5	5	5	5
TM (filtered)	0	0	5	0	5	0
VOX	2	2	2	0	2	0
AOX	2	2	2	0	2	0
Complex organics	1	1	0	0	1	0
P/CP	2	2	2	0	2	0
RO	2	2	2	0	2	0
Toxicity	0	0	0	0	0	0

Table 6.3 - Sampling requirements for Runs 2a and 2b, 27-28 November 1990 (numbers of samples at each site)

Determinand	Site 1	2	3a	3b	4a	4b
RC	16	16	0	0	0	0
RM	16	16	0	0	0	0
NRM	8	8	0	0	0	0
TM (total)	0	0	0	0	0	0
TM (filtered)	0	0	0	0	0	0
VOX	0	0	0	0	0	0
AOX	0	0	0	0	0	0
Complex organics	0	0	0	0	0	0
P/CP	0	0	0	0	0	0
RO	0	0	0	0	0	0
Toxicity	0	0	0	0	0	0

Table 6.4 - Sampling requirements for runs 3 and 4, 4-5 December and 11-12 December 1990 (Number of samples at each site)

Determinand	Site 1	2	3a	3b	4a	4b
RC	24	24	12	12	12	12
RM	24	24	12	12	12	12
NRM	5	5	5	0	5	0
TM (total)	5	5	5	0	5	0
TM (filtered)						
VOX	2	5	2	0	2	0
AOX	2	5	2	0	2	0
Complex organics						
P/CP	2	5	2	0	2	0
RO	2	5	2	0	2	0
Toxicity	2	2	2	0	2	0

Hurlbert (1984) has discussed the problems inherent in upstream-downstream studies of the type in which a bioassay such as the *G. pulex in situ* test is likely to be used. 'Pseudoreplication' occurs in these experiments because it is impossible to allocate treatments randomly. Bioassays are placed either above or below the relevant discharge without the ability to control for other variables such as substrate type, flow rate, or temperature. It is thus impossible confidently to ascribe the cause of any observed changes between upstream and downstream stations to the effects of the discharge.

Stewart-Oaten *et al* (1986) have devised an experimental design that may overcome this problem in many environmental impact studies. They argue that the detection of the effect of a discharge can be achieved by testing whether the difference between underlying mean abundances or responses at control and impact sites changes once the discharge begins. Samples replicated in time are taken Before the discharge begins and After it has begun at both the Control (upstream) and Impact (downstream) sites (BACI). These data are then used to estimate the trends of the response before and after the impact commences and standard statistical techniques can be used to see if these trends differ significantly.

Field cages were prepared and filled with food material and adult male *G. pulex* obtained from the River Darenth, North Kent, as described in an earlier report (Crane and Maltby 1990). The deployment of the technique was staggered over a five-day period, both before and after the commencement of disinfection. The correct use of the BACI approach would normally require samples to be spaced separately in time to a far greater extent than in this study, in order to assess long-term effects. However, it was felt that the short-term approach described here would give a valuable indication of effects within a clearly circumscribed time frame.

On day one of the 'Before' exposure period (4 November 1990), one holding basket, containing 18 *G. pulex* and four leaf weight control cages, was deployed at a station upstream from Porthtowan STW. A similarly filled holding basket was deployed simultaneously at a downstream station. The upstream station was located approximately 100 m above and the downstream station approximately 100 m below the STW outfall. On days two, three, four and five of the Before

study, further pairs of baskets were deployed at the stations above and below the STW, so that on day five there were a total of five baskets at the upstream station, and five baskets at the downstream station.

Retrieval of the baskets was also staggered. The pair deployed on day one were removed on day six (10 November), and the remaining pairs were each removed six days after deployment, on days seven, eight, nine and ten respectively. The temperature of the water was taken each time a pair of cages was deployed and retrieved. After retrieval, live *G. pulex* and any fragments of leaf remaining in the cages were removed and rinsed in tap water until free of attached sediment. Their weight was then determined after drying at 60 °C to constant weight.

The same exercise was repeated after the commencement of disinfection on 28 November 1990. The first pair of holding baskets were deployed above and below the sewage treatment works on 29 November and retrieved six days later. The fifth pair was deployed on 3 December and retrieved on 9 December.

Calculation of feeding rate and analysis of results

Gammarus feeding rate was calculated from Equation 1

$$C = \frac{((L1*C1)-L2)}{W*D} \qquad \text{Equation 1}$$

where

C = feeding rate (mg of leaf/mg animal/day)
L1 = initial weight of food material (mg)
L2 = final weight of food material (mg)
W = weight of *G. pulex* (mg)
D = the number of days of deployment (six in these experiments).

C1 is the leaf weight correction factor calculated from the cages without *G. pulex* and was included to allow for changes in leaf weight caused by factors other than *G. pulex*. C1 was calculated for each site for both deployment periods from Equation 2.

$$C1 = \frac{\text{Sum of Final food weight/Initial food weight}}{\text{Number of samples (20 in each experiment)}} \qquad \text{Equation 2}$$

If C1 differed significantly between sites, the different correction factors were used for their respective sites. If no significant difference in leaf weight change could be found between sites then a mean C1 was applied similarly for both sites.

The feeding rates calculated from Equation 1 were transformed to stabilise the variances using log(x+1). They were then analysed in two separate ways:

1. The five temporal replicates at each site were grouped and analysed using ANOVA for both Before and After deployments, a procedure that mimics the traditional method used for determining upstream and downstream differences in environmental impact assessments (Green 1979).

2. The BACI approach was adopted. The mean response was calculated for each temporal replicate and the difference between upstream and downstream pairs was found. After testing for non-additivity with a Turkey test, the Before and After disinfection deployments were compared with a t-test, using the difference between the upstream and downstream response as input parameters. All procedures were performed according to Stewart-Oaten *et al* (1986) and Sokal and Rohlf (1981).

Mortality was analysed in a similar way to feeding rate. A generalised linear model was used to analyse differences in overall mortality between upstream and downstream sites on the Before and After deployments respectively. A t-test was then used to compare the differences in mortality after treating the data according to the BACI method.

6.6 Results and discussion

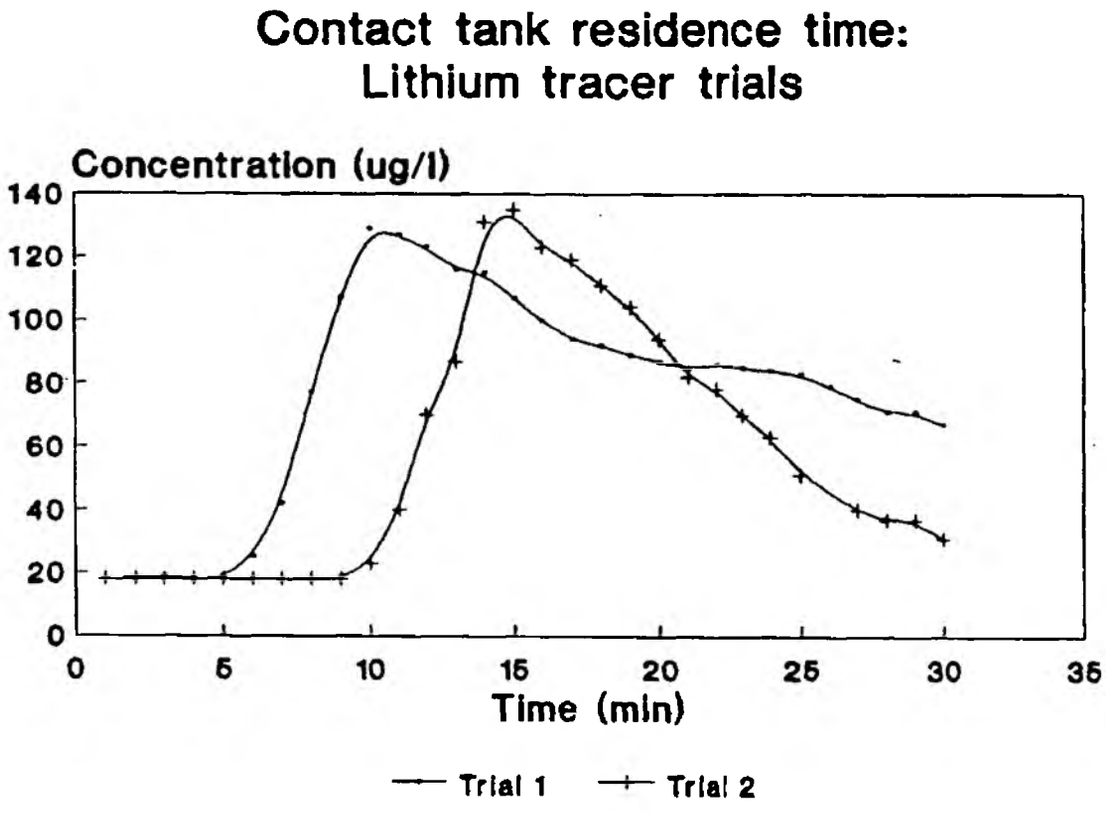
6.6.1 Contact tank performance trial

Both trials indicated that the residence time of sewage in the tank exceeded five minutes (Figure 6.3). In neither case was lithium detected above background levels within this period, although the lag only just exceeded five minutes during the first trial but exceeded ten minutes during the second trial. The stock solution of lithium salt was of similar concentration in both trials, and the concentration of Li in the effluent from the tank peaked at between 120-130 $\mu g\ l^{-1}$ on each occasion. It is likely that the volume of fluid in the tank was similar on both occasions, but that the initial flow rate during the first trial was higher than during the second. There was a notable difference in the rate of decline in Li concentrations between the two trials. Given that the initial volume of tracer solution was similar in each case, mass balance considerations suggest that there was a substantial reduction in flow rate during the course of the first trial; as a consequence, Li was eliminated from the system only slowly and remained at concentrations well above background after 30 minutes. In contrast, during the second trial the greater part of the Li introduced was eliminated within 30 minutes, suggesting that flow during the latter part of the trial was greater than during a comparable period in the first trial. Measured flow rates support this interpretation. Flow was 4-8 litres s^{-1} during the first trial, decreasing with time, and 3-13 litres s^{-1} during the second trial, increasing with time. The contact tank appeared therefore to be achieving the required residence time, although the combinations of flow and residence actually measured do not permit conclusions to be firmly drawn with regard to flow rates in excess of 10 litres s^{-1}.

Extending the contact time artificially had variable effect of bacterial kill. In general, there was only a slight improvement in kill, but in samples where the initial kill rate was poor, the additional contact time effected improvements of between 0.3 and 1 log unit.

In summary, it was clear that the contact tank was providing the minimum required contact time of five minutes with greater consistency than had been

Figure 6.3 Contact tank performance trial - lithium concentrations in outflowing sewage



the case during previous trials at Trevaunance Cove. Contact time and minimum retention time are not the same thing, however, and the trials showed very marked differences in the overall retention times of lithium which suggested that either resident tank volume or flow rate varied rapidly with time. The available data do not provide information on effective contact time at flow rates higher than 10 litres s^{-1}.

The dosing equipment did not appear to be able to match changes in sewage flow rate adequately, since concentrations of PAA and peroxide in the outflowing sewage and rivers were frequently high and were very variable. This indicated that substantial quantities were 'unused' even when bacterial kill rates were high.

6.6.2 Sewage chemistry

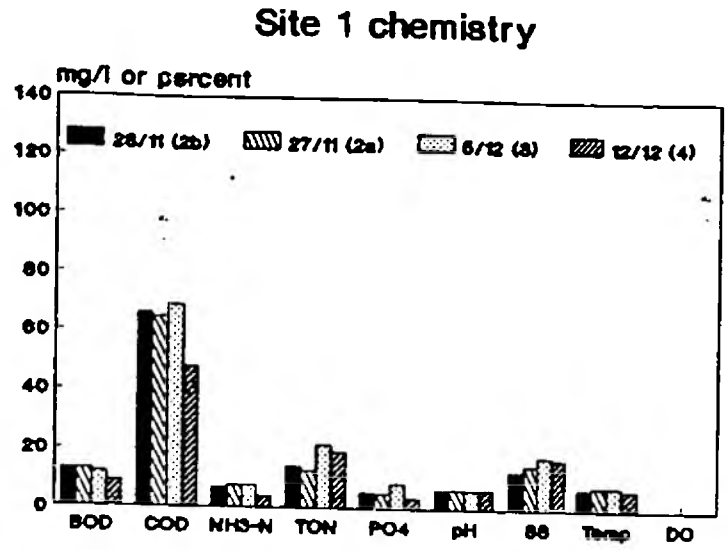
The variation in average values of routine chemical determinands is summarised for all sites and trials in Figures 6.4 and 6.5. Most determinands were present in concentrations which were consistent within each site over all trials. Between-trial variability was highest for BOD, COD, and SS at Site 2; this was to some extent a consequence of the effect of different PAA doses.

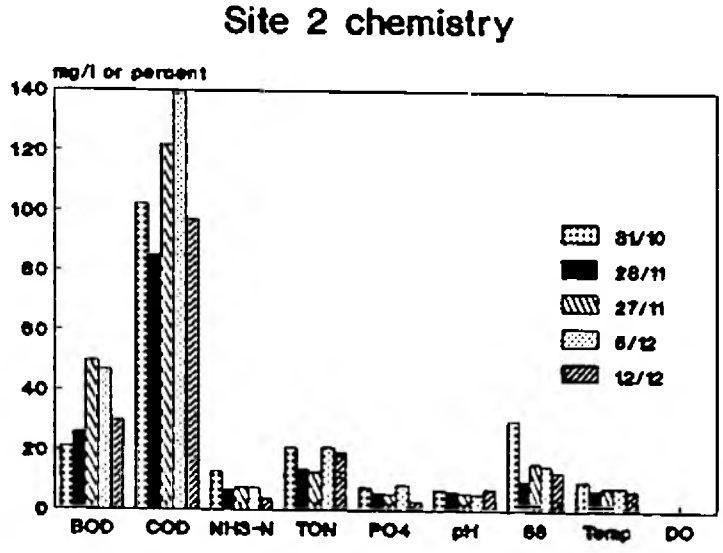
6.6.3 River chemistry

COD concentrations followed a similar trend at sites 3a, 3b, and 4b (river sites remote from discharge), presumably reflecting the effects of variation in erosion within the catchment. Dissolved oxygen levels followed a similar trend at Sites 4a and 4b; levels at 4b were generally lower than at 4a, however, and it is likely that the reductions observed at these two sites were attributable (to some extent at least) to different causes.

The major effects of disinfection on river quality are illustrated in Table 6.5. BOD and COD at point 4a are expressed as a percentage of the values in pre-contact-tank sewage. It is clear that both BOD and COD were substantially higher during the fixed-dose trials than during the pre-disinfection baseline trial.

Figure 6.4 Routine chemistry for sites 1,2 and 4a. Values shown are 24 hour mean values for the dates given





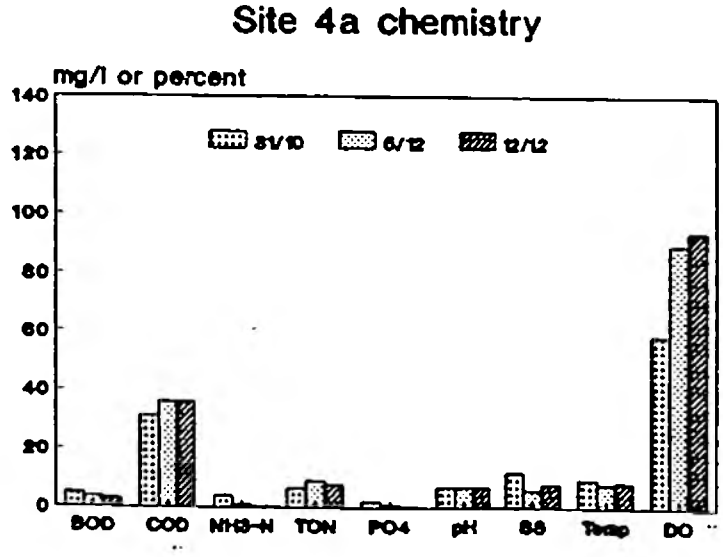
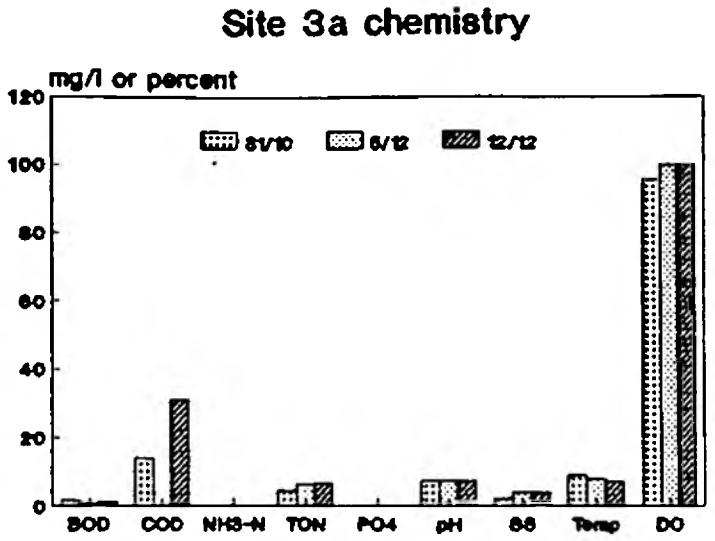
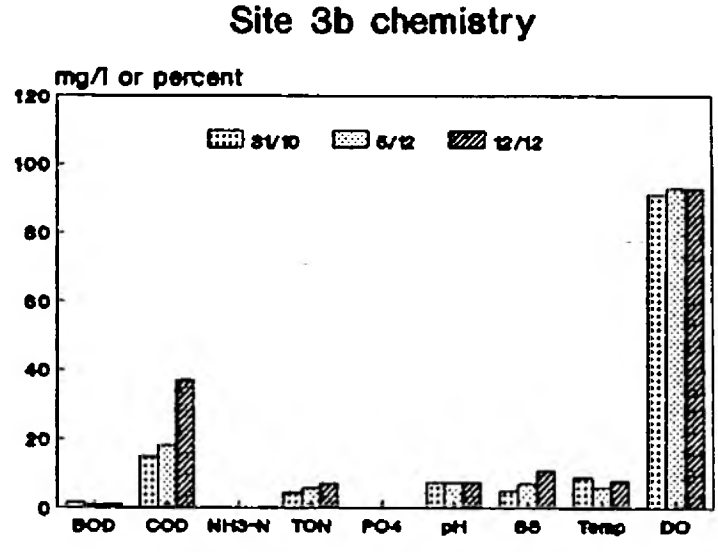
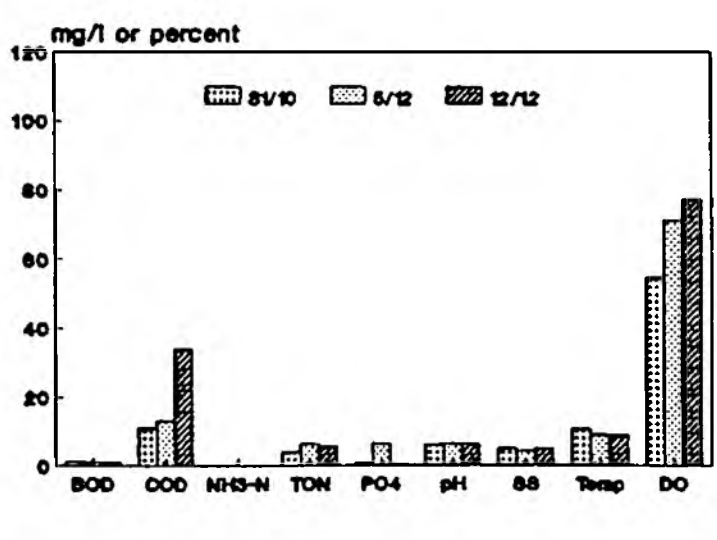


Figure 6.5 Routine chemistry for sites 3a, 3b and 4b. Values shown are 24 hour mean values for the dates given







Ammonia and phosphate were consistently present in concentrations above background levels at Site 4a; these could be attributed to the sewage discharge, and implied a dilution factor of at least 5.

Trace metal levels were very consistent between trials. In general, the sewage discharge did not contribute significantly to the levels already present in the river. Most notable was the very large contribution made to concentrations downstream by an unmonitored input, presumably some form of mine drainage. This input appeared to be responsible for a substantial reduction in water quality.

Table 6.5 - Effects of disinfection on river quality (BOD and COD at point 4a as a % of values at point 1)

Determinand	Trial 1 (30/10)	3 (5-6/12)	4 (11-12/12)
BOD	23	31	30
COD	30	52	75

6.6.4 Effects of PAA on sewage chemistry

The main effect of PAA addition to the sewage was a very large increase in BOD and COD (Figure 6.6, Table 6.6). The increase appeared to be related to nominal PAA dose, with larger increases at 6 mg l^{-1} than at 4 mg l^{-1}. Changes in phosphate, pH and suspended solids were less consistent; the first increased in three of four trials while the latter two decreased in three of four trials. The increase in pH recorded on the final trial was unexpected, and was associated with an unusually wide variation in values at Site 2; this result should therefore be treated with some caution.

Figure 6.6 Percentage change in routine chemical determinands after disinfection

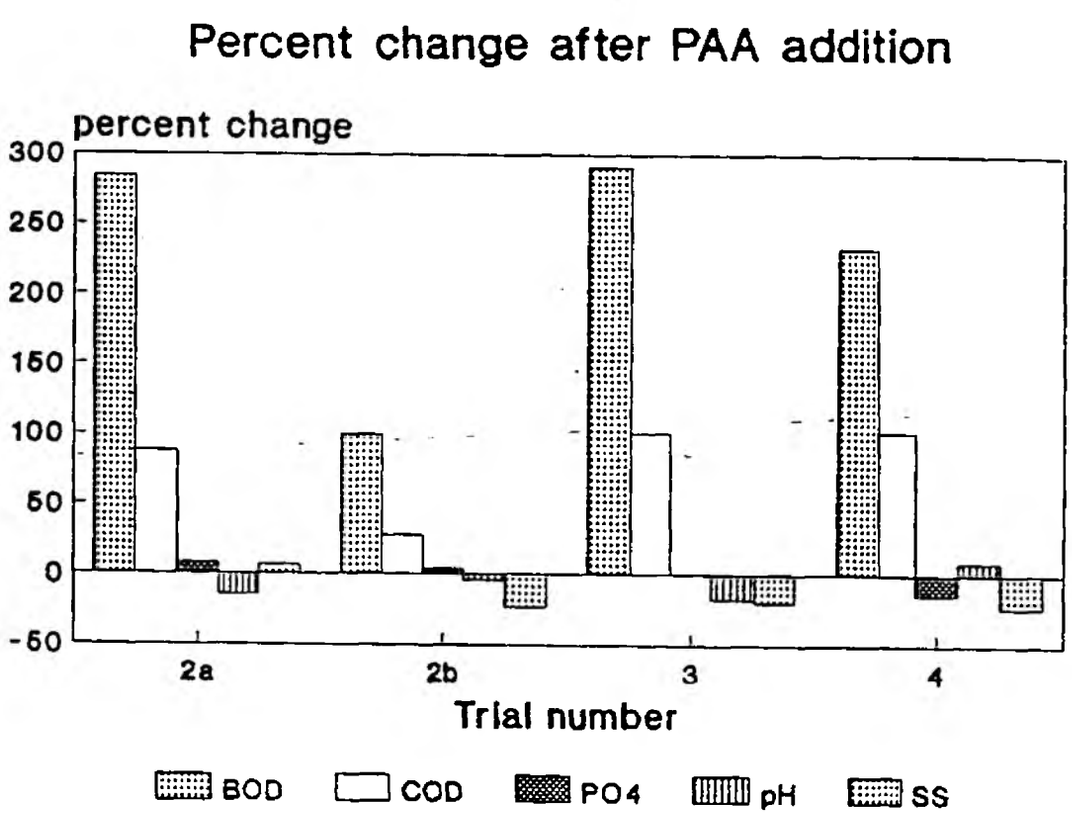


Table 6.6 - Percent change in routine chemical determinands after disinfection

Run	BOD	COD	PO_4	pH	SS
2a	284	88	7.7	-14.9	6.7
2b	100	28	3.7	-4.5	-23.0
3	291	101	1.1	-17.8	-20.0
4	233	102	-14.5	8.9	-23.0

The effects of PAA on trace metal partitioning are summarised in Figures 6.7, 6.8 and 6.9. There was a consistent increase in the fractions of Cu, Zn, Cd and Pb in soluble form, although the increases were generally small. These changes are not surprising, since PAA will tend to break down organo-metallic complexes.

There is little evidence of phenolic compounds or VOXs in most samples; most of the determinands listed were close to, or below, the detection limits on most occasions. AOX concentrations were not unusual. There was no evidence for any of the organic compounds of an effect of PAA. GCMS analysis for complex organics revealed compounds typical of domestic sewage, and provided no evidence for any effect of PAA disinfection on this group of determinands.

6.6.5 Effectiveness of applied doses of PAA in disinfecting sewage

As noted above, the concentrations of residual oxidants in the receiving water indicated that nominal doses may have been exceeded on occasion by a considerable margin.

There was no apparent relationship between measured sewage variables and bacterial kill rate. In general, most determinands varied relatively little over each trial, while bacterial kill rate varied widely. While it remains possible that variation in kill rate might be due to some unmeasured variable, two measured factors had sufficient capacity for variation to merit consideration: dose rate and flow rate.

Figure 6.7 Concentraion of Copper at each site

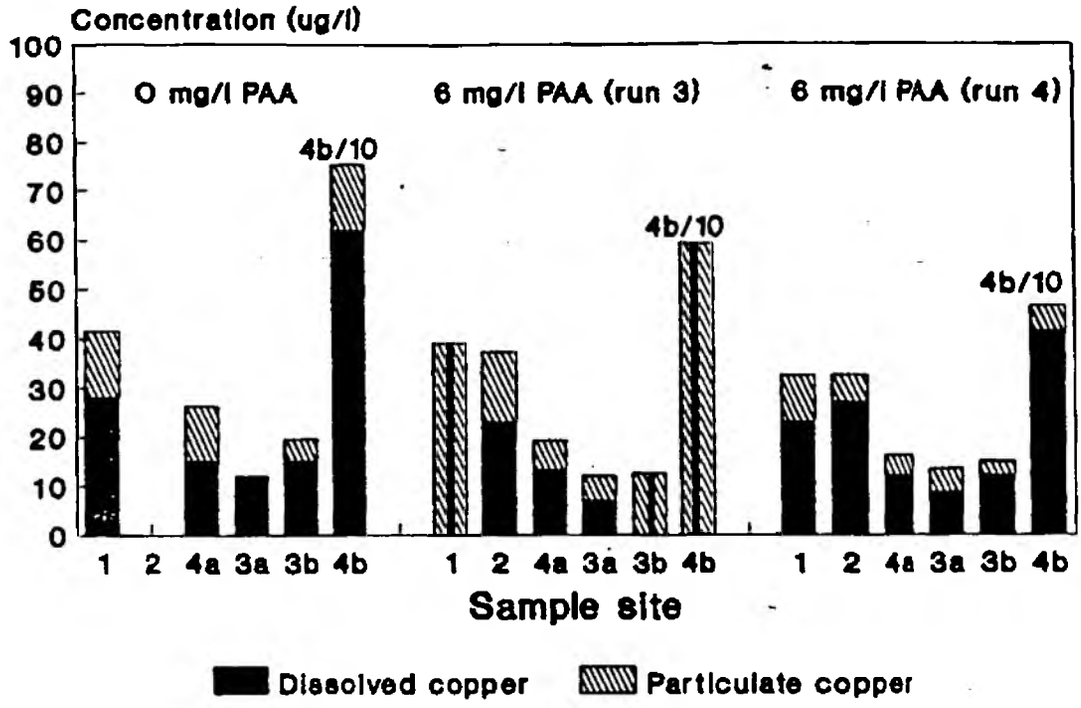


Figure 6.8 Concentration of Zinc at each site

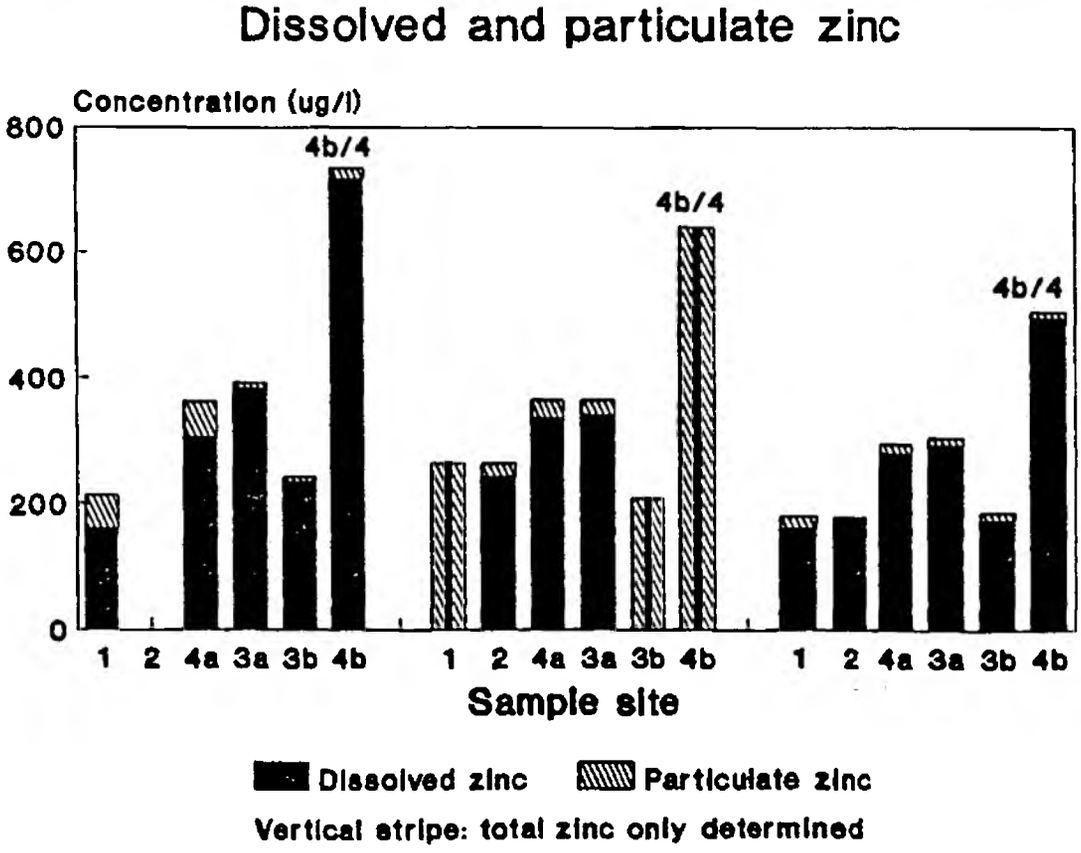
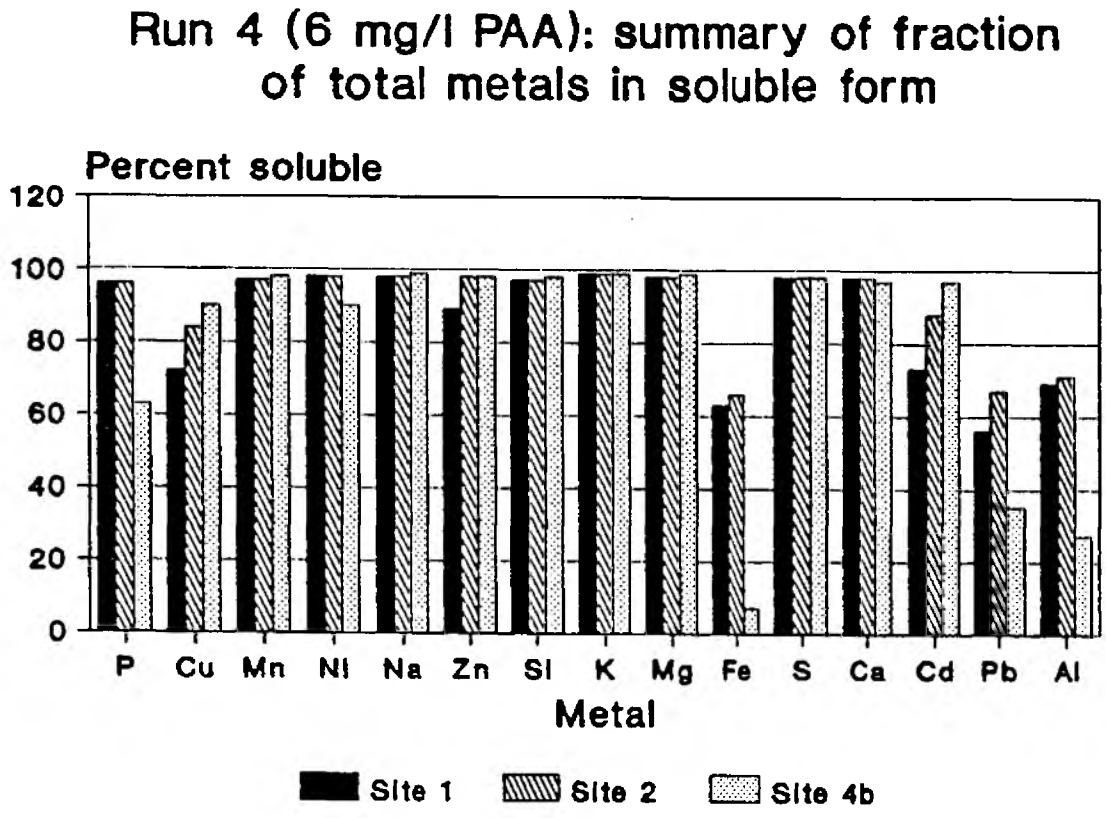


Figure 6.9 Percentage of total metals in soluble form in fixed dose trial run 4



Since flow rate often exceeded the measurement scale and bacterial numbers often fell below (or were very close to) the detection limit (LOD), the following approach was adopted:

1) Bacterial samples from Site 2 were classified as greater than the limit of detection (>LOD) or less than the limit of detection (<LOD). These correspond to classes of "complete" or "partial" kill respectively;

2) The median flow rate of sewage was calculated for each class;

3) The median flow for each nominal dose was plotted against the percentage of samples for a given trial in which the numbers of bacteria at Site 2 were >LOD (Figure 6.10).

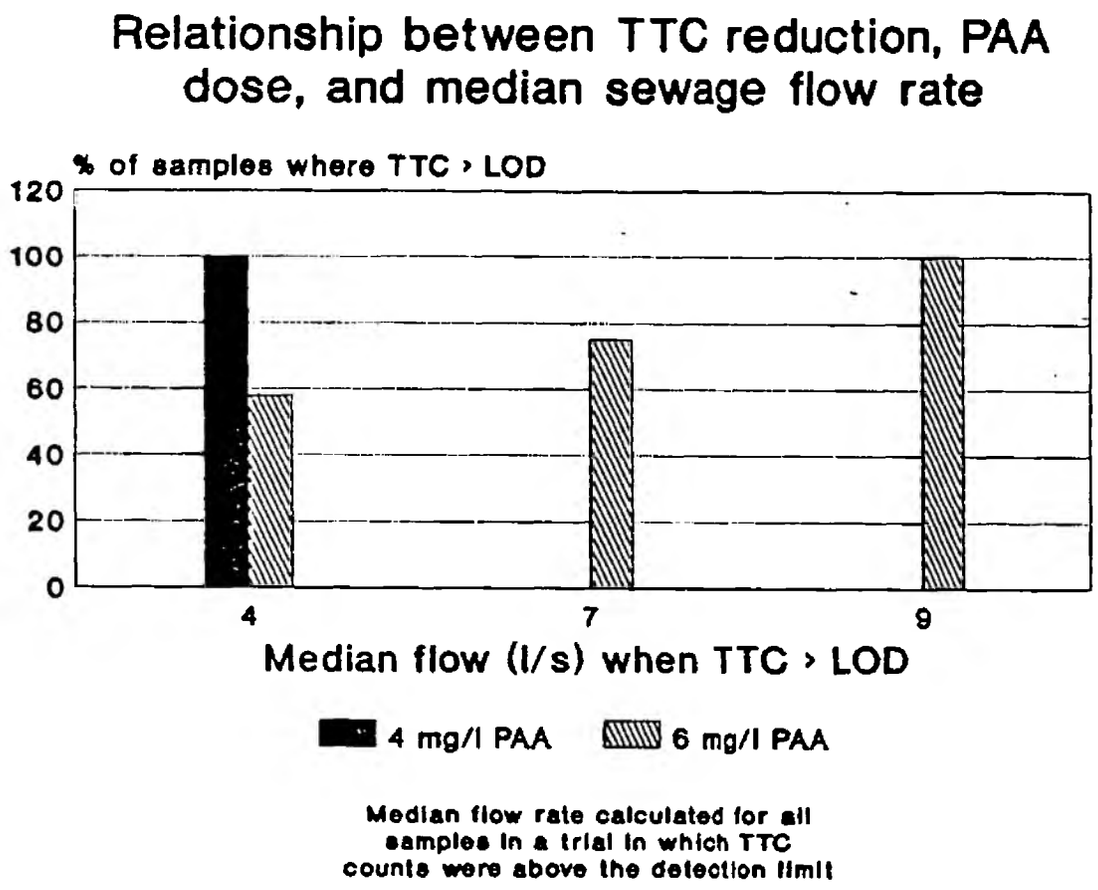
This analysis indicated that, in general, kill rate was lower at higher flow rates and/or at lower dose rates. Table 6.7 indicates the median flow rates associated with the two classes of effect, as well as the median flow rate over each trial.

Table 6.7 - Relationship between median flow rate and frequency of samples in which bacterial counts were less than or greater than the limit of detection (flow in l/s)

Run	Date	Dose	Median flow when TTC<LOD	Median flow when TTC>LOD	Median flow over run	% of samples where TTC>LOD
1	30/10	0	-	-	3.00	-
2a	27/11	6	2.5	7.0	3.75	25
2b	28/11	4	-	4.0	4.00	0
3	4/12	6	2.5	4.0	3.25	42
4	11/12	6	-	9.0	9.00	0

Since actual dose rates may have fluctuated widely, it would be useful to have available some index of actual dose. Hydrogen peroxide is less reactive than PAA, and concentrations in the outflow from the contact tank were examined as a

Figure 6.10 Relationship between median flow rate (in litres per second) and frequency of samples in which bacterial counts were greater than the limit of detection



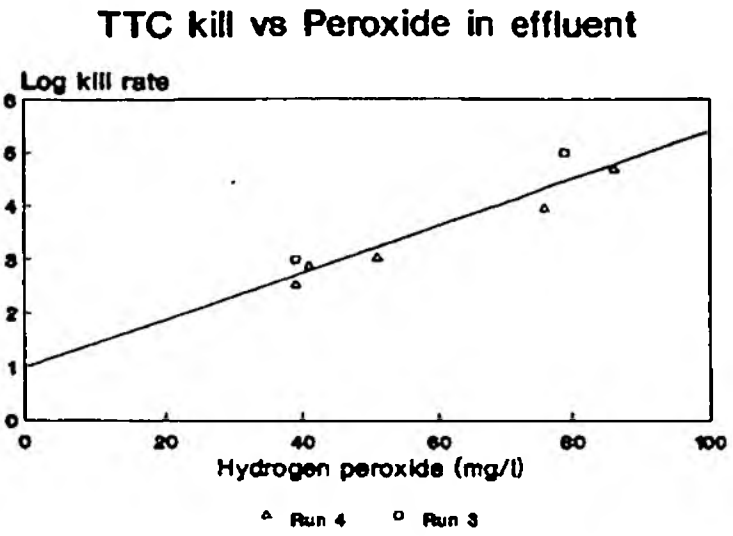
possible marker for actual dose. Peroxide concentrations at Site 2 were therefore plotted against TTC log kill rate for Trials 3 and 4, when the nominal dose rate was 6 mg l^{-1} . There was a clear positive relationship (Figure 6.11), suggesting that despite the apparent overdose, there was some link between the amount of disinfectant actually administered and bactericidal activity.

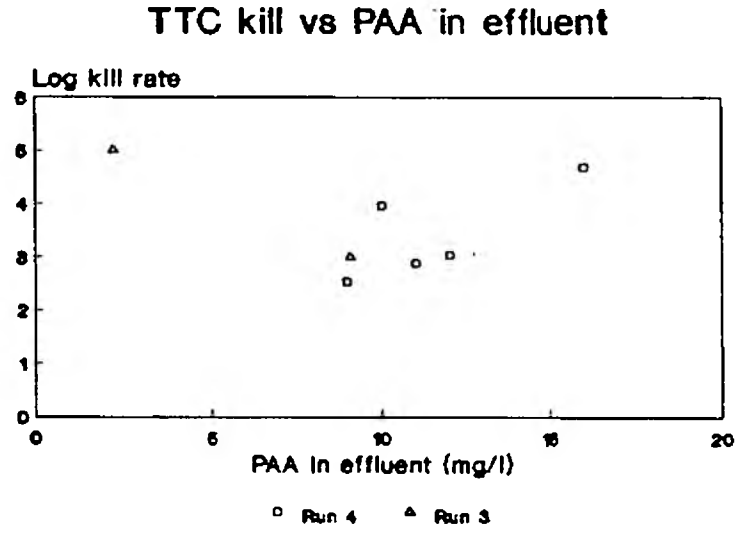
The relationships between PAA and peroxide concentrations and between PAA concentrations and bacterial kill rate were also examined (Figure 6.11). These relationships were generally good, and reflected approximately the ratio of PAA to peroxide in the disinfectant formulation. One data point was a clear outlier, however; this was the sample collected at Site 2 at 0600 on 5 December. In this sample, PAA concentration was low, but was associated with a high peroxide concentration and with a very high 'organic bromamine' concentration. These observations are consistent with much of the PAA having been used up in oxidising organic matter in the sewage. Field notes record that this sample was 'thick', although this was not reflected in routine chemical analysis. The high concentration of 'organic bromamine' would be expected if a substantial amount of organic material had been oxidised by PAA.

There is evidence that three factors (flow, dose and organic material) affected the efficacy of disinfection, although insufficient data are available to draw firm conclusions. Peroxide may be a useful marker for dose level.

The results of analysis for 'non-routine' organisms indicated that the duplicating laboratory consistently estimated higher numbers for F^+ coliphage, *Pseudomonas* and *Salmonella*, but detected enterovirus and rotavirus less frequently. There is therefore a considerable degree of uncertainty associated with the estimates provided, and the effects of the disinfectant are difficult to quantify. Only F^+ coliphage and *Pseudomonas* were detected with high frequency in samples. The effects of disinfection are summarised in Table 6.8, from which it can be seen that the proportion of organisms killed was frequently very low in comparison to the effects reported above for TTC and FS.

Figure 6.11 Comparison of the log kill rate for thermotolerant coliforms with the concentration of PAA in the effluent





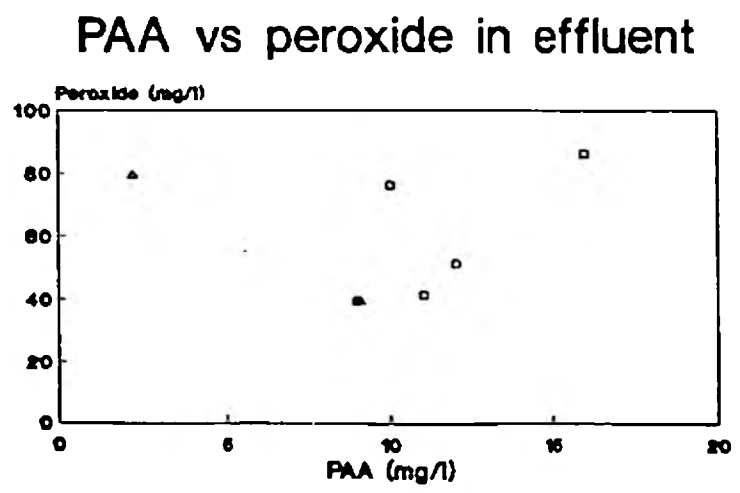


Table 6.8 - Summary of the effects of disinfection on pathogenic bacteria and viruses in sewage samples (% reduction) (M=main laboratory, D=duplicating laboratory)

	Trial							
	2a		2b		3		4	
Determinand	M	D	M	D	M	D	M	D
Rotavirus								
Enterovirus	=		=		=		=	
F_+ coliphage		>80		>50	=	>40	?	>95
Pseudomonas		>>>		>40	>>>	30	>50	>94
Staphylococcus					>90		>>>	
Campylobacter		>>>						
Salmonella				=	>90	>>>		>90

>>> - numbers reduced to below limit of detection
= - no clear effect on numbers
> - percentage reduction greater than indicated value

F^+ coliphage and *Pseudomonas* both appeared in river water samples. The latter was detected during the final trial (11/12 December) only, although abundance in sewage was relatively higher during the previous trial (5/6 December) when it was not detected in the river.

6.6.6 Toxicity of sewage and river water

The patterns of toxicity reported were fairly uniform between Trials 3 and 4. In both trials, river water samples collected at 0900 were more toxic than those collected three hours earlier; this difference may be due to the period for which samples were held prior to testing.

Most of the toxicity observed in undisinfected sewage and the upstream river site can adequately be accounted for by the levels of dissolved trace metals present. The increase in toxicity observed in disinfected sewage and in the receiving water downstream of the discharge must have been due either to changes in chemistry effected by the disinfectant or to some component of the disinfectant itself. The changes in metal partitioning noted above were too

small to account for the increases in toxicity, and there is little evidence either of significant concentrations of organic compounds or of any changes in these following disinfection. It seems therefore likely that the disinfectant itself was the toxic agent in this case.

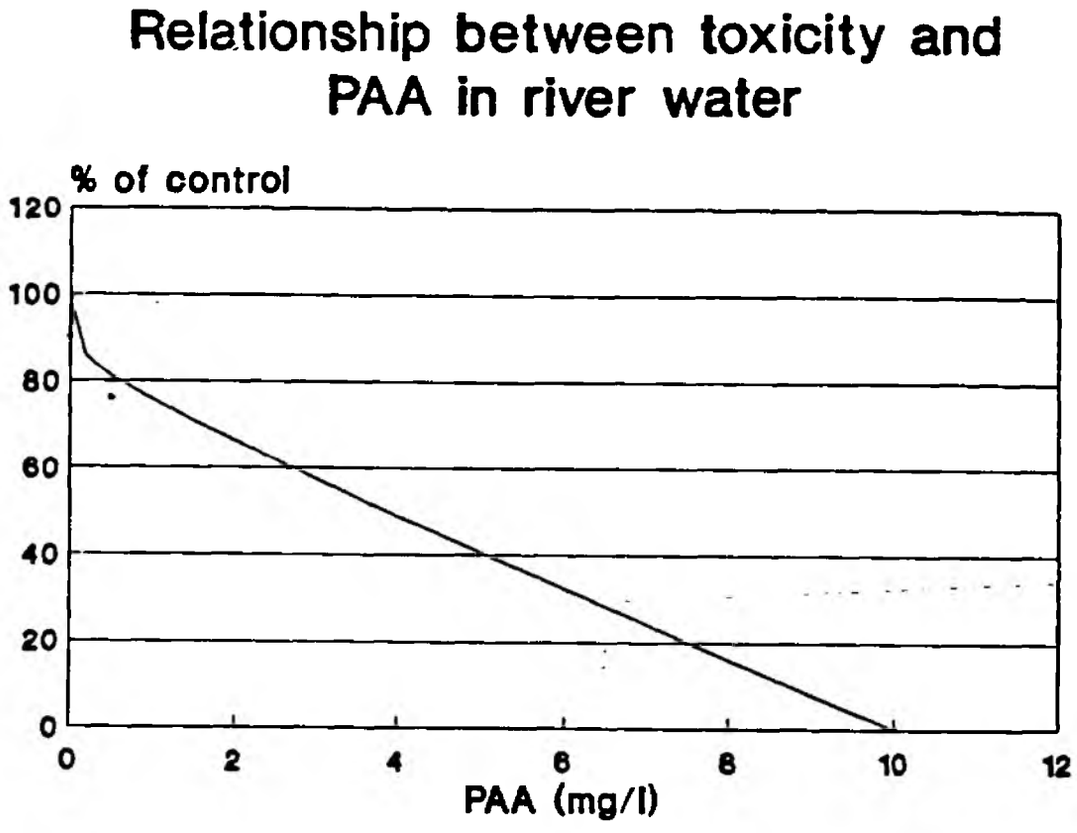
An approximation of the toxicity of PAA can be obtained by examining the relationship between PAA concentration in sewage and river water samples and the percentage normal development of oyster embryos in corresponding samples at 10% concentration (Figure 6.12). Normal development was completely arrested at an effective concentration of 1 mg l^{-1} and reduced by 24% at an effective concentration of 0.05 mg l^{-1}. It is unlikely that all of the toxicity observed is due to PAA alone, however, and peroxide levels in both river and sewage samples were consistently high.

6.6.7 Effects on Gammarus

The results from the *in situ* deployment of *G. pulex* suggest that the Porthtowan River is subject to variable water quality over short time periods. The significantly higher mortality of animals deployed at the upstream site during the Before trial indicated the presence of toxicity which can in no way be related to the Sewage Treatment Works. This toxicity did not persist, and *G. pulex* deployed at the same site one month later did not suffer the same level of mortality.

The feeding rate of the *G. pulex* was very low at both upstream and downstream sites, before and after disinfection. This low feeding rate may have been related to the time of year, or to poor water quality at both sites. Although the data suggested that disinfection may have had a beneficial effect upon the feeding rate of *G. pulex*, a rigorous statistical analysis (the BACI method) demonstrated that the variability of the response in this study was too high between treatments to be able to ascribe any significance to these results. The high mortality at the Before upstream site was a confounding factor that may also have affected the measurement of feeding rate, by eliminating the most sensitive individuals.

Figure 6.12 Relationship between PAA concentration in river samples and the percentage normal development of oyster embryos in corresponding samples at 10% concentration



6.7 Conclusions on PAA trial at Porthtowan

Peracetic acid disinfection of Porthtowan sewage was generally effective during fixed-dose trials, but there was evidence that the nominal dose levels were not accurately maintained at all times. Substantial concentrations of peroxide were observed in river samples, and sewage fungus present in the river appeared to be largely eradicated during the trials.

Sewage chemistry was measurably affected, with substantial increases in BOD and COD, and small increases in trace metal soluble fractions. VOX, AOX and phenol compounds were not major sewage constituents, and did not appear to be affected by the addition of disinfectant.

Increased BOD and COD levels in sewage were reflected in higher levels in the river. Effects on trace metals in sewage were not reflected to a significant extent in river samples. The influence of mine drainage on river quality was large in comparison to the effects of disinfected and undisinfected sewage.

Limited evidence suggests that sewage flow rate, disinfectant dose and organic matter in sewage may all contribute to disinfection efficiency. Disinfection appears to be less effective at low doses and higher flow rates, and the presence of sufficient organic matter may consume PAA and lead to the formation of brominated by-products. Hydrogen peroxide concentrations in disinfected sewage provided a useful indicator to the effectiveness of bacterial kill.

Although the kill rate for TTC and FS was generally good, there is no comparably clear pattern for non-routine bacteria and viruses. F^+ coliphage did not appear to vary in numbers in any manner related to changes in viral numbers. Percentage kill, where it could be estimated, appeared to be highly variable. Most probably, kill rate was highly variable, but quantitative data were very limited.

Disinfected sewage proved highly toxic to both oyster embryos and Microtox, but there were insufficient samples available to determine whether toxicity was related in any way to effectiveness of bacterial kill.

The Porthtowan River did not prove to be an ideal site for an investigation into the *in situ* effects of sewage disinfection with PAA. There is evidence to suggest that one or more toxic substances occur episodically above the Sewage Treatment Works, and this makes it difficult to assess the instream effects of either disinfected or undisinfected sewage effluent. This study has shown that PAA disinfection did not significantly increase the mortality or reduce the feeding rates of *G. pulex* over a series of six-day deployments. However, it must be emphasised that this was a site- and temporally-specific investigation with a very limited relevance to other running water systems, or even the Porthtowan system at different times of the year, when water temperature and flow rate will differ. It is important to examine the *in situ* response of *G. pulex* under several different conditions of PAA dosing before it is possible to assess the risk that the disinfection process presents to this species.

7. EVALUATION OF METHODOLOGY USED IN ENUMERATION OF VIRUSES AND PATHOGENIC BACTERIA IN DISINFECTION FIELD TRIALS

7.1 Introduction

This section is a review of non-routine microbiological analyses conducted in all 1990 trials. Table 7.1 summarises the results and compares the techniques of the non-routine microbiological analyses carried out under this contract.

An initial request to both laboratories for details of their methods produced information that was not definitive, so that their methods could not be fully compared. Subsequent requests for greater detail did yield more usable information.

In general terms, the methods used are those developed for the food industry or for clinical examinations and they have not undergone extensive development or evaluation for environmental use, where interferences may be different, such as seawater salts, organisms, or numbers of organisms that do not occur in other circumstances. Also, the inclusion of a pre-enrichment step to some extent allows environmentally stressed organisms to recover.

In environmental samples where there are complex interactions, the time delay between sampling and examination may have considerable influence on the numerical value of the result. This is particularly so where the relationships between organisms may have been altered, as in disinfection studies.

The use of Most Probable Number (MPN) methods can only be considered to give a result (depending on dilutions etc.) to the correct order of magnitude. However, colony or plaque counting methods will be subject to technical inaccuracies, interpretation and the sensitivity of the system and therefore the degree of accuracy may not be much better. In microbiology measurements are usually made so that a several orders of magnitude increase in the numbers of the organism occurs in the examined volume, or large differences are observed in comparisons.

Table 7.1. Comparison of techniques and results of non-routine microbiological analyses

	WALLACE - EVANS				SEVERN - TRENT				
Organism	Pre-enrichment	Presumtive to level	Confirmation to level	Count type	Pre-enrichment	Presumtive to level	Confirmation to level	Count type	Similarity of labs
Salmonellae	yes	genus	genus**	mpn	yes	genus	genus**	mpn	good
Pseudomonas	yes	genus	species	mpn	no	species	no	direct	poor^^
Campylobacter	yes	genus	genus**	mpn	no	genus	no	mpn	moderate
F+ coliphage	-	na	na	direct	-	na	na	direct	good
Staphlococcus	yes	species	species	mpn	no	species	no	direct	poor
E coli	yes	species	species	direct	not requested				
Faecal Strep.	no	species	no*	direct	not requested				
Rotavirus									good
Enterovirus									good

* Media very specific confirmation not usually practiced
** Species identification not requested
^^ Media with widely differing properties used

Neither laboratory volunteered details of any Quality Control system.

Usual microbiological techniques apply selective pressures within the growth media which select the desired organism and its close relatives to grow - the presumptive test. For more precise identification to species level further specific tests are applied - the confirmation tests. By the nature of these tests the confirmed level cannot exceed the presumptive level and would normally be lower.

7.2 Comparison of techniques and results from the laboratories

7.2.1 Salmonellae

Severn-Trent (ST) returned more positives or higher levels of positives. This may well be an effect of lack of confirmation. Wallace - Evans (WE) set their upper limit of dilutions rather low and therefore returned a high proportion of "greater thans".

7.2.2 *Pseudomonas*

The pattern of presence or absence (PA) is similar in the returns from both labs, but the levels from ST are much higher than those from WE; probably due to lack of confirmation and use of differing media.

7.2.3 Campylobacter

Not detected by either lab in compared samples.

7.2.4 Staphylococcus

Considerable difference in the media used by the two labs. Always present at Trevaunance in the duplicated samples and the pattern of PA at Porthlevan was

similar. High levels reported by ST may be a function of lack of confirmation but low upper detection limit from WE meant that all their results are ">", and they do not to have seem to have learnt from earlier difficulties.

7.2.5 F+ coliphage

The PA pattern from both labs was similar. Numerically ST were higher but many WE results were ">". Again, the difficulties encountered with previous samples do not appear to have been learnt from.

7.2.6 Rotavirus

Only one of the compared samples reported as positive (1 litre).

7.2.7 Enterovirus

For the available comparable results at Trevaunance, ST showed a much higher proportion of positives (7/12) than WE (0/12) at a mean level of 15 pfu per litre. At Porthtowan WE consistently reported a few per litre but ST report none per 10 litre. These results suggest changes in technique during the period between the two investigations, but since the picture is reversed a more likely explanation is that changes in the cell lines have occurred that have not been detected by QA.

7.3 Summary

WE methodology is more precise, but dilution ranges have not been wide enough with the result that there are too many ">" reported. ST have reported some apparently precise numbers, but this is marred by a lack of confirmation techniques. Neither lab has produced excellent results. The results of each individually must be qualified by the foregoing comments and direct comparisons cannot be made other than in the use of PA comparisons.

8. OVERALL CONCLUSIONS

Sections 4.5 and 4.6 list the detailed conclusions that arise out of the field trials using ultraviolet light as a sewage effluent disinfectant. Broadly UV looks a promising method for disinfecting secondary effluent, though there is a need for more work on photoreactivation of microbial pathogens in field conditions and, in common with the other trials on PAA, the study has demonstrated the need for work on non-routine microbiological analysis techniques as they relate to water.

Sections 5.7 and 6.7 contain the detailed conclusions of the work on PAA. The Trevaunance Cove studies did not suggest that PAA performed well as a disinfectant of fine screened sewage, while the Porthtowan work suggested that PAA was generally effective at reducing numbers of indicator micro-organisms. However, it questioned the ability to accurately monitor and maintain PAA at required concentrations.

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