CSIR DIVISION OF WATER TECHNOLOGY

HEALTH PROGRAMME

ASSESSMENT OF WATER QUALITY PROBLEMS DUE TO MICROBIAL GROWTH IN DRINKING WATER DISTRIBUTION SYSTEMS

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FINAL REPORT

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CONTENTS

EXECUTIVE SUMMARY

The purpose of a water distribution system is to supply the consumer with potable water and to ensure good hygienic sanitary conditions. However, this is not always the case. Although potable water is disinfected prior to entering the distribution system, the manner or type of disinfection used is not always suitable for maintaining the water quality. It is known that breakthrough may occur which results in the regrowth and aftergrowth of organisms in the distribution system. The occurrence of bacterial aftergrowth in water distribution systems has been well documented.

Biofilms consist of immobilised cells which do not wash out as rapidly as their suspended counterparts. This results in the accumulation of relatively large cell populations within the distribution system. Biofilms have also been recognized as complex microbial ecosystems consisting of various microorganisms, *e.g.* diatoms, algae, filamentous and rod shaped bacteria, yeasts, fungi, and micro-invertebrates. All these microorganisms are known to cause a deterioration in taste, odour and appearance of drinking water. Biofilm formation also effects the bacteriological quality of potable water. The presence of opportunistic pathogens such as *Pseudomonas, Mycobacterium* and *Klebsiella* in biofilms may pose a threat to public health. *Legionella pneumophila* was found to proliferate in hot water systems.

The aims of the study have been as follows: (i) to conduct an in-depth study on biofilm composition and formation in a potable water distribution system; (ii) to study the effect of biofilm formation on the quality of the final drinking water, and (iii) to assess the extent of biofilm formation and evaluate the possible risk to human health.

Total coliforms were isolated from 33% of the samples tested. Ten percent of the samples contained more than 5 total coliforms per 100 mℓ, which is above the maximum allowable limit as recommended by Watertek. Faecal coliforms were isolated from 10% of the samples tested and counts were found to vary from 0 to 7 CFU per 100 mℓ. No faecal coliforms are allowed per 100 mℓ of drinking water. Twenty-five different fungal cultures were isolated from the distribution system and counts fungal counts were found to vary between 0 and 3 755 org. per 100 mℓ. Most of the organisms (95%) isolated were identified as members of the genus *Bacillus*. Other organisms isolated were identified as *Staphylococcus, Micrococcus, Acinetobacter, Flavobacterium, Aeromonas, Klebsiella, Citrobacter* and *E. coli*. *Legionella* bacteria were also isolated occasionally.

Bacterial numbers counted in the distribution system of apartment buildings were found to be much higher than in private houses. The age of the building and of the plumbing system was found to play an important role in the potable water quality of the building. Substantially higher bacterial counts were found in water samples collected from older buildings. The bacterial population of biofilms were found to concur with the sessile population found in the water samples. Mild steel was found to be the best supporter of bacterial growth in water distribution systems and copper the best inhibitor. Higher corrosion tempos were found in relatively older buildings than those found in relatively new buildings.

LIST OF APPENDIXES

1. INTRODUCTION

The purpose of a water distribution system is to supply the consumer with potable water and to ensure good hygienic sanitary conditions. The water distribution system can therefore be considered as a channel in which the final water is distributed to the consumer's home. Such a channel should be free of any adverse effects on the quality of the water, *i.e.* the water consumed should be of a similar quality to that of the water leaving the waterworks. However, this is not always the case. Although potable water is disinfected prior to entering the water distribution system, the manner or type of disinfection used, does not always provide for maintaining a desired water quality. It is known that breakthrough may occur which results in the regrowth and aftergrowth of organisms in the distribution system. The occurrence of bacterial aftergrowth in water distribution systems has been well documented (LeChevallier *et al.*, 1982; LeChevallier & McFeters, 1985; McFeters *et al.*, 1986; Van der Kooij, 1982).

Drinking water contamination, resulting from the breakthrough of microorganisms, remains one of the difficulties faced by producers of potable water world-wide. This eventually, as already mentioned, leads to the regrowth of bacteria, resulting in biofilm formation. Biofilm formation within the water distribution system has not been well studied and little definitive information is available on the many factors influencing microbial growth and survival (Reasoner, 1988). The study of microbial behaviour and the relationships within the distribution system are therefore challenging and complex.

Biofilms consist of immobilized cells which do not wash out as fast as their suspended counterparts. This results in the accumulation of relatively large cell populations within the distribution system. It has been established that on average, there are as many as 10 000 sessile organisms for every planktonic organism present in recirculating water. Biofilms have also been recognized as complex microbial ecosystems consisting of various microorganisms, *i.e.* diatoms, algae, filamentous and rod shaped bacteria, yeasts, fungi and micro-invertebrates (Hinzelin & Block, 1985). All these microorganisms are known to cause a deterioration in taste, odour and appearance of drinking water (Victoreen, 1974).

The formation of a biofilm in a water distribution system may disturb waterflow and

intensify corrosion of the materials used in the construction of the system. Sulphate reducing bacteria were identified as the single most causative organisms responsible for most of the corrosion in distribution systems (Von Holy, 1987; Bondonno, 1990). These bacteria play a dual role in causing corrosion. They initially act as cathodic depolarisers after which corrosive hydrogen sulphide is produced (Von Holy, 1987). Other microorganisms directly involved in corrosion include the sulphur-oxidizing bacteria and iron bacteria. Algae and fungi are indirectly involved in corrosion as these organisms are involved in biofilm formation. Biofilm formation also affects the bacteriological quality of potable water (Geldreich *et al.*, 1972). A threat to public health may also be posed by the presence of opportunistic pathogens such as *Pseudomonas aeruginosa, Mycobacterium kansassi* and *Klebsiella pneumoniae* in biofilms. Other opportunistic pathogens *i.e. Legionella pneumophila* were found to proliferate in hot water systems (Grabow *et al.*, 1991)

The formation of a biofilm will only take place if an energy source (organic or inorganic hydrogen) and other compounds required for the synthesis of biomass are present in sufficient amounts. Bacteria growing in the water distribution system, particularly those contributing to the colony counts of potable water, were found to be chemoheterotrophic (Van der Kooij *et al.*, 1982). Berg and co-workers (1981) also found bacteria growing under low nutrient conditions to be more resistant to disinfectant actions than bacteria growing under high nutrient conditions. *Escherichia coli* has been shown, when attached to a clean glass surface, to be 11 times more resistant to free chlorine than unattached cells (Grabow, 1989).

The Health Programme of the Division of Water Technology (Watertek) undertook to research and assess microbiological growth in the water reticulation and distribution system of a major metropolitan area. The aims of the study have been as follows: (i) to conduct an in-depth study on biofilm composition and formation in a potable water distribution system; (ii) to study the effect of biofilm formation on the quality of the final drinking water; (iii) to assess the extent of biofilm formation with reference to possible risk to human health.

2. RESEARCH APPROACH

A detailed literature survey has been completed using the available services (WATERLIT & SARIS) supplied by the Division of Information Technology, CSIR and data received from overseas experts in the field. Attempts have been made to address different aspects of microbial growth in potable water distribution systems.

In an attempt to assess the problems that might arise from microbiological growth in the water distribution and reticulation systems, questionnaires (Appendix A) were compiled and sent out to 25 municipalities and water boards throughout South Africa. They were selected according to the size of their distribution system. In an attempt to ascertain the existence and extent of complaints about taste and or odours in a major metropolitan area, an in-house questionnaire (Appendix A) was compiled.

Due to logistical problems it was decided to concentrate, for the purpose of this study, on one metropolitan area only. Water and biofilm samples were collected at random over a three year period (January 1988 - December 1990) from private houses, apartment buildings, and other institutions. Apartment buildings were considered to be high rise buildings with one or two central reservoirs serving the whole building. An attempt was made to include sampling points in both "old and new" suburbs, thereby facilitating comparison studies between private houses and apartment buildings, as well as comparison studies between relative old and new buildings. Sampling points were also selected in a manner which ensured a correlation study between water quality (microbiological, chemical and physical) and distance from the water source or reservoir.

Various piping materials were also studied in order to evaluate their relative support to biofilm formation. The final water quality and biofilm formation and composition were also evaluated in order to facilitate correlation studies between the quality of the potable water and extent of regrowth. A modern approach to biofilm monitoring is the development of replaceable sampling surfaces which allowed for more detailed observations of relatively undisturbed portions of the biofilms. An example of such a sampling system is the Pedersen's device (Fig. 1). This device will be described in full in paragraph 3.4.

3. MATERIALS AND METHODS

3.1. Microbiological analyses

Grab samples were collected at the various sites in sterile two litre Nalgene bottles and analyzed within six hours of collection. Two millilitres of a 30% sodium thiosulphate solution were added to each bottle to neutralize any chlorine present at the time of collection. Water samples were analyzed for total coliforms, faecal coliforms, injured coliforms, fungi, actinomycetes and total and heterotrophic plate counts. The membrane filtration technique (APHA, 1985) was used for the enumeration of total and faecal coliforms, injured coliforms and fungi. Culture media used included dehydrated m-Endo agar Les, m-FC agar, m-T7 agar (LeChevallier *et al.*, 1982) and Potato dextrose agar (pH 3,5) (Hinzelin & Block, 1985) respectively. Total and heterotrophic plate counts were done according to APHA (1985) using Total plate counting agar and R2A agar respectively. Actinomycetes were enumerated using Actinomycetes isolation medium, Czapek's agar, Chitin agar and Starch-casein agar (Waksman, 1961; Williams *et al.*, 1989).

A number of water samples were also tested for the presence of opportunistic pathogens such as *Legionella, Klebsiella, Aeromonas* and *Pseudomonas aeruginosa*. *Legionella* bacteria were enumerated using a Buffered charcoal yeast extract agar (BCYE) according to Grabow *et al.* (1991). In short, an appropriate volume of the sample was filtered through a membrane (pore size = 0,45 µm). The membrane was placed in a wide necked bottle containing 10 mℓ of sterile tap water, and sonicated for 10 min to release the bacteria from the membrane. Quantities of 0,1 mℓ of this concentrate or dilutions thereof were streaked on the BCYE agar plates. These plates were incubated for three to five days at 37° C, after which they were examined for the presence of *Legionella* by staining smears of these colonies with a commercially available antibody conjugate specific for *L. pneumophila* and *L. micdadei* (Zeus Technologies, Inc.).

Ampicillin dextrose agar (Havelaar *et al.*, 1987) was used for the enumeration of *Aeromonas*. A modified McConkey agar (MCIC) (APHA, 1985) was used for the

enumeration of *Klebsiella*. The mPA-B medium, as recommended by Havelaar *et al.* (1985), was used for the enumeration of *Pseudomonas aeruginosa*.

3.2. Chemical/physical analyses

Grab samples were collected in sterile one litre Schott bottles. Parameters tested (APHA, 1985) included: iron, zinc, sulphate, potassium, magnesium, chloride, hardness, alkalinity, total suspended solids (TSS), dissolved organic carbon (DOC), total organic carbon (TOC) and conductivity. The Lovibond 2000 comparator MK.II (Tintometer GMBM, West Germany) was used to determine free and total chlorine on site. Results were confirmed in the laboratory, using the DPD Ferrous Titrimetric method (APHA, 1985). Temperature (°C) was determined on site, while pH was determined in the laboratory at room temperature. The Langelier corrosion index was also calculated (Langelier, 1936).

3.3. Assimilable organic carbon assay

The assimilable organic carbon (AOC) assay, as developed by Van der Kooij and co-workers (1982), was applied. Test water samples were pasteurized, cooled and inoculated with a small number of test organisms from a stock culture of *Pseudomonas fluorescens* strain P-17. The inoculated water samples were incubated at 15°C and sampled repeatedly to establish the maximum density (N_{max}) of test organisms in the water samples. Organisms were enumerated as colony forming units (CFU) using the spread plate technique. The CFU values were converted into AOC concentrations using an empirically derived yield factor. The yield factor (µg AOC per CFU) was determined by adding known concentrations of acetate to a test water sample. Linear regression was used to estimate the relationship between the density of the CFUs and acetate concentration after correcting for any AOC present in the water before adding acetate.

3.3.1. Preparation of stock culture

The method, described by Van der Kooij and co-workers (1982) was used for the preparation of a stock culture of *Pseudomonas fluorescens* strain P-17. Precultures were prepared by inoculating 50 mℓ sterile tap water, containing one mg/ℓ acetate, with a turbid suspension of a 24 hour old cell culture, grown on a Lab Lemco slant. With this inoculum, an initial colony count of ±500 CFU/mℓ was introduced. Incubation at 25° C for one week results in $4x10^6$ CFU/mℓ. The precultures were then stored at 4° C and used to inoculate the test water samples.

3.3.2. Preparation of glassware

Biological oxygen demand (BOD) bottles were thoroughly cleaned to remove any traces of AOC from the glassware. Cleaning was achieved using chromic acid, and alternative rinses with hot tap water and a 10% nitric acid solution. Graduated pipettes (1 mℓ) were also cleaned with chromic acid and repeatedly rinsed in streaming tap water. The pipettes were then heated overnight at 250° C before use. Cotton plugs were not used.

3.3.3. Preparation of water samples

Clean, sterilized BOD bottles were carefully filled at selected sampling points. Care was taken to minimize contamination of the water by pipe materials, sediments or dust. After the samples were returned to the laboratory, all vegetative bacterial cells in the water were killed by pasteurization at 60° C for two hours. After cooling, samples were inoculated with precultured cells of *P. fluorescens* strain P-17 and incubated at 15±0,5° C without shaking until a maximum cell concentration (N_{max}) was reached. Growth was measured daily by plate colony counts, using Tryptic soy agar. Colony forming units were counted after a 48 hour incubation period at 25±0,5° C. All growth experiments were carried out in triplicate.

3.4. Biofilm formation and corrosion tempos

A modified Pedersen's biofilm reactor (Fig. 1) was used to determine the aftergrowth potential of microorganisms and biofilm development on different supporting materials (Pedersen, 1982). This unit is constructed of cast iron and contains replaceable coupons (76mm x 25mm x 1mm), manufactured from different materials. The coupons can be removed individually without draining the system. The 4'6-diami-dino-2-phenylindole (DAPI) fluorescing stain method, as described by Wolfaart *et al.* (1990) was used to determine the numbers of sessile microorganisms colonizing the surface of the different material coupons. Biofilm composition was determined by sonicating the coupons in 10 mℓ sterile water for 10 min, after which samples were plated on R2A agar. Representative colonies were then picked off and identified. Each sample was also tested for the presence of sulphate reducing bacteria. Total corrosion tempos (CR) for each of the materials used were determined according to Mittelman & Geesey (1987).

3.5. Biotoxicity and mutagenicity assays

Bioassays were carried out according to standard procedures as described by the United States Environmental Protection Agency (EPA, 1985), using waterfleas (*Daphnia pulex*). A LC50 was determined after 24 and 48 hours. The method described by Ames and co-workers (1975) as modified by Kfir and co-workers (1982) was used for the detection of mutagens in water. Mutagenic activity is reported as a mutation ratio (MR value), which is a calculated ratio between the formation of mutant colonies and spontaneous mutation levels.

3.6. Identification of microorganisms

Bacterial colonies, representing the total population on each plate were maintained on Tryptic soy agar slant after isolation and purification, using standard microbiological techniques. Samples from the enrichment studies were streaked out on Tryptic soy agar to obtain single colonies. Gram stains were performed on heat-fixed smears of

24 hour old cultures. The staining method was carried out as described by Holdeman *et al.* (1977). The results were confirmed with the lysis of cells in a two percent (m/v) potassium hydroxide solution (Gregerson, 1978). Cell morphology was determined by a phase contrast examination of wet mounts (Pfennig & Wagener, 1986) and bright field microscopy of Gram stained preparations. Endospore formation was determined on one week old cultures, grown on Tryptic soy agar slants, as well as a sporulation medium. Bright field microscopic examinations of Schaeffer-Fulton stained smears (Doetsch, 1981) were carried out, as well as, phase contrast examinations of wet mounts. The sporulation medium consisted of (g/l): peptone, 5; meat extract, 3; $MnSO_4 \cdot 4H_2O$, 0,005; agar, 15.

Further physiological characteristics, needed for the final identification of the microbial isolates (Krieg & Holt, 1984), were done according to Gerhardt *et al.* (1981).

3.7. Statistical analysis of the results

Results were analyzed using the personal computer package Statgraphics 4.0 (STSC, 1989).

4. RESULTS AND DISCUSSION

4.1. Literature survey

A detailed literature survey has been completed and is attached as an appendix (Appendix D) to this report. The study reviews the following topics: the source of microbial growth in the system; the water distribution layout with reference to structure and materials used; factors affecting microbial growth in the system with special reference to total organic carbon levels and disinfection; health-related aspects of the microbial growth and the microbial quality of the final drinking water. The literature study indicated the merit of investigating the extent of microbial growth in water distribution systems in South Africa.

4.2. Questionnaires

Twenty-five questionnaires were sent out to various water suppliers, of which only 16 were completed and returned. Seven of the 16 questionnaires answered, indicated that taste and odour problems do exist. Only one water supplier identified the taste and odour problem positively as being due to bacterial growth. One water supplier indicated geosmin and another an excess chlorine smell as the reasons for the taste and odour complaints. According to the water suppliers, taste and odour problems were treated either by relocating intake water from one place to another, or by flushing the hydrants until a residual chlorine was measured. Activated carbon filtration was also used.

The water purification treatment performed by all water suppliers, consisted of a flocculation/coagulation process. Pre-chlorination followed by flocculation was applied by four of the water suppliers. A sedimentation process, using lime and ferrichloride, was used by only one. All water suppliers surveyed, performed filtration followed by chlorination of the final water. Residual chlorine concentrations reported in the final water ranged from 0,02 mg/ℓ to 1,5 mg/ℓ. However, most of the returned questionnaires (56%) indicated a residual chlorine concentration between 0,1 and 0,2 mg/ℓ. Reported bacterial counts for water in the distribution system ranged for total coliform counts between 0 to more than 1000 CFU per 100 mℓ and for standard plate counts from 0 to 100 CFU per 1 mℓ.

Sixty-nine individuals, representing 38 different suburbs, participated in the in-house questionnaire. Thirty-three percent complained about taste and odour problems. Only two individuals reported the problem to the authorities. Fifty-eight percent of the complaints were due to taste (6 - chlorine; 13 - "other") and 17 to odour (5 - chlorine; 12 - "other").

Data collected from both questionnaires clearly indicates that bacterial aftergrowth occurs in water distribution systems of most of the country's major metropolitan centres. It also shows that the treatment processes do not always meet the requirement to maintain a safe microbiological water quality as specified by the SABS (SABS, 1984) and recommended by the CSIR (Kfir, 1989). However, it must be stated

that this assumption is based on the occasional monitoring of the water quality as supplied to the final user and might be a once only situation.

4.3. An evaluation of the extent of biofilm formation in private houses.

During the first phase of this study (January 1988 - December 1989), biofilm samples were collected from several private houses in various suburbs in one of South Africa's major metropolitan areas. The first set of samples were collected from two houses situated in two relatively new suburbs. Members of the local municipality assisted in the sampling process by opening pipes leading to the houses. Biofilm samples were collected from the inside walls of the pipe and tested for the presence of various microorganisms. Microbiological analyses performed were total coliforms, faecal coliforms, *Klebsiella*, *Aeromonas*, *Pseudomonas* and *Legionella*. The potable water was not tested. Although heavy background growth occurred, none of the specific organisms tested could be enumerated. The residual chlorine concentration for both sampling points was less than 0,2 mg/ℓ. A second set of samples were then collected from older suburbs. Sampling procedures and microbiological analyses used were similar to those used during previous studies, except for the implementation of an enrichment step in buffered peptone water and nutrient broth before streaking onto selective media. Although bacterial growth occurred in the enrichment media, none of the microorganisms tested for, using different selective media, could be enumerated. In both studies no chemical/physical analyses were included.

As a result of the unsuccessful attempts to directly isolate any health-related microorganisms from the scrapings collected from the reticulation system, it was decided to collect samples from underground pipes believed not to have been opened in 40 years. Water samples and scrapings from the inside walls of the pipes were collected from two different locations in a relatively older suburb. Sampling procedures were the same as for the previous study. Results are summarized in Tables 1 and 2. No chemical or physical parameters were included in this study. Although heavy background growth occurred in both types of enrichment media of the potable water samples collected from house no. 1, none of the opportunistic pathogens tested could be enumerated. Similar results were found for enrichment studies of scrapings

collected from the pipe.

Total coliforms and faecal coliforms were enumerated from enrichment media of scrapings collected from house no. 2. None of the opportunistic pathogens, including total and faecal coliforms, could be enumerated. Potable water samples collected from house no. 2 were also negative for any of the organisms selected. However, a total plate count of 177 CFU per mℓ was found in the potable water collected from house no. 2, compared with the 19 CFU per mℓ counted in potable water collected from house no. 1. These results confirmed biofilm formation in the water distribution and reticulation systems in this area. Results (heavy background growth) from samples collected in other suburbs of the same metropolitan area also confirm biofilm formation. However, no pathogenic or opportunistic pathogens could be isolated. As this was the first initial stage of the research project, this could be due to improper enrichment of the samples rather than to a total absence of these organisms from the distribution system.

4.4. **An evaluation of the potable water quality and the identification of microbial growth in the reticulation system.**

In an attempt to identify problem areas for further studies, it was decided to evaluate the water quality in different suburbs in the same metropolitan area used in previous studies. Eighty different water samples were collected from apartment buildings, private houses and other institutions during the period January 1989 to December 1990. Microbiological analyses performed on these samples included total coliforms, faecal coliforms, filamentous yeast and fungi, standard plate counts and heterotrophic plate counts. Before testing for the presence of *Klebsiella*, *Aeromonas* and *Pseudomonas* species, water samples were enriched using Tryptic soy broth. All chemical and physical parameters were tested for, as has already been described.

Total coliform counts varied from 0 to 26 CFU per 100 mℓ (Fig. 2) and were isolated from 33% of the samples tested. Ten percent of the samples contained more than 5 total coliforms per 100 mℓ. Injured coliform counts varied from 50 CFU per 100 mℓ to 850 CFU per 100 mℓ. The relatively high injured coliform counts, compared with the

total coliform counts, indicate that these bacteria, although present in the distribution system, cannot always be detected using conventional methodology. These bacteria are injured due to the treatment processes and are in need of special favourable conditions. By using the m-T7 agar one caters for such favourable conditions (LeChevallier *et al.*, 1982). Such favourable conditions can also be found in the reticulation system, resulting in regrowth of total coliform bacteria. The detection of injured coliforms in drinking water provides a more sensitive and accurate database that can assist in finding microbiological problems at an earlier stage (McFeters & Singh, 1988). Injured coliforms can also be of public health significance, since opportunistic pathogenic bacteria are more resistant to injury than coliforms. Opportunistic pathogens reported to be enumerated using the m-T7 agar are *Klebsiella, Pseudomonas, Aeromonas, Citrobacter, Pasteurella, Acinetobacter* and *Flavobacterium* (Skadsen, 1990).

Faecal coliforms counts varied from 0 to 7 CFU per 100 mℓ (Fig. 3) and were isolated from 10 % of the samples tested. Heterotrophic plate counts varied from $1,65 \times 10^3$ to $6,59 \times 10^6$ (Fig. 4) and were found to be 2 logs higher (geometrical mean) than the standard plate count (Figs. 4 & 5). Fungal counts were found to vary between 0 and 3 755 organisms per 100 mℓ (Fig. 5). Various types of imperfect fungi were isolated from water distribution systems in recent years (Rosenzweig & Pipes, 1988). It has been shown in laboratory studies that fungal spores and mycelia can be inactivated by low concentrations of chlorine, but survive in some habitats within the water distribution system. Of 38 chlorinated drinking water samples analyzed by Hinzelin & Block (1985), 50 % were found to be contaminated by yeasts and 81 % by filamentous fungi. Most of the samples tested in this study were also found to be contaminated with filamentous fungi only. Elevated storage tanks open to the atmosphere appear to be a significant source of fungal input to the water distribution system (Rosenzweig & Pipes, 1988).

Members of the actinomycetes were isolated from two samples only, collected in one of the suburbs studied. Unfortunately these actinomycetes cultures could not be further identified. Members of the actinomycetes are known to produce secondary metabolites such as 2-methylisoborneol (2-MIB) and geosmin, causing an earthy-musty taste and odour in water (Gerber, 1983). This normally occurs under conditions of oxygen stress.

Many reservoirs have been shown to be troubled by the production of these two metabolites (Aoyama, 1990).

Most of the organisms (95 %) isolated from the enrichment studies were identified as members of the genus *Bacillus* (aerobic, Gram-positive endospore-forming bacilli). This is to be expected, as all the water samples tested were exposed to treatment processes which should eliminate most other vegetative cells. Endospores are resistant to treatment processes and can easily enter the water distribution system, where they can multiply. The genus *Bacillus* are known to contain species pathogenic to humans and animals (Starr *et al.*, 1981). Other Gram-positive organisms were found to be cocci. These organisms were identified as members of the genera *Staphylococcus* and *Micrococcus*.

Most of the Gram-negative organisms isolated were identified as *Pseudomonas fluorescens*. *Pseudomonas aeruginosa* was never isolated from any of the samples tested. Other Gram-negative organisms isolated were identified as members of the genera *Acinetobacter, Flavobacterium, Aeromonas, Klebsiella, Enterobacter* and *Citrobacter*. *Escherichia coli* was isolated only once from samples collected from an apartment building. These results are similar to those found by other researchers. Families most frequently isolated by Gambasinni and co-workers (1990) were Bacillaceae (57,6% of total population), Micrococcaceae (43,9% of total population) Pseudomonadaceae (40,9% of total population) and Enterobacteriaceae (22,7% of total population).

Members of the genus *Legionella* were the only organisms isolated using selective media. These organisms were isolated from both houses and apartment buildings. No trend was found for any type of building. *Legionella* counts were found to vary from between 36 and 231 organisms per 100 mℓ. These organisms are also known to grow in close association with blue-green algae and other chemolitotrophic organisms. Their presence may therefore confirm the presence of biofilms in the water distribution systems of the metropolitan area studied.

Twenty-five different fungal cultures were isolated and purified from the water samples tested. These organisms were found to be members of the genera *Aspergillus,*

Penicillium, Fusarium, Phialophora, Curvularium and *Mucor*. The genera *Aspergillus, Curvularium, Penicillium* and *Fusarium* are known to contain human pathogens (Wilson & Plunkett, 1967; Marcus *et al.*, 1991). It is also known that fungal spores may cause allergic reactions in some humans upon consumption. The pathogenic yeast, *Candida albicans* was not isolated from any of the water samples tested. However, although yeasts other than *Candida albicans* were found in this study, they were not recorded or identified.

Results of the chemical/physical parameters are summarized in Table 3 and Fig. 7. All parameters were found to comply with the SABS standards (maximum permissible limit) for drinking water (SABS, 1984; Kempster & Smith, 1985). Seven percent of the samples tested contained more than 0,03 mg/ℓ iron (Fe). Zinc concentrations were found to vary from less than 25 µg/ℓ to 625 µg/ℓ. Total chlorine levels varied between 0 and 0,2 mg/ℓ. No residual chlorine was recorded with only one exception where 0,01 mg/ℓ was measured. A minimal residual chlorine concentration of 0,2 mg/ℓ is needed for effective disinfection.

No correlation was found between bacterial counts and the chemical/physical parameters of the various houses and apartment buildings. Similar results were found by Bondonno (1990). However, bacterial counts were found to correlate with chemical/physical parameters when sampling was conducted along the distribution system on the same date. A correlation coefficient of -0,9487 was found between bacterial counts and total chlorine concentration in the water. This indicates that the higher the chlorine concentration, the lower are the bacterial counts recorded. Correlation data of other parameters are given in Table 3. The high correlation found between hardness and conductivity does not necessarily indicates a direct relation between these two parameters and bacterial counts.

4.5. A comparison of the water quality collected from apartment buildings and private houses.

The difference in the bacterial water quality of apartment buildings and private houses was evaluated. The results (Fig. 9 - 13) clearly indicate the water quality in apartment

buildings to be of a lower standard than that of private houses. Bacterial counts (heterotrophic plate count, total plate counts and fungi) were 2 log or more higher in apartment buildings (Fig. 11 - 13). All faecal coliforms isolated during the second phase of this study were isolated from water samples collected in apartment buildings. Of the total coliforms isolated, 79% were isolated from apartment buildings. This can probably be ascribed to the presence of a central reservoir in apartment buildings which supplies the whole building with water. Maintenance of such reservoir is often neglected. During the collection of samples for this study, two reservoirs were found standing open on top of the buildings. This may result in the contamination of the plumbing system by dust and organic matter such as leaves and birds. The water quality of apartment buildings analyzed during this study, although not repeatedly analyzed, does not comply with the SABS limits for drinking water in general. A total plate count of 222 CFU/mℓ (geometrical mean) was found for apartment buildings, compared with the 57 CFU/mℓ (geometrical mean) for private houses.

4.6. **Biotoxicological studies.**

Bioassays were carried out on all the water samples. Only one sample collected from a school showed a 100% *Daphnia* lethality within 24 hours. A second sample, and samples from surrounding houses were immediately collected and analyzed. Results showed a 40% lethality after 24 hours and a 100% lethality after 48 hours for the second sample collected. The remaining samples showed a lethality of less than 10% after 48 hours. A lethality of less than 10% is interpreted as an absence of toxicity.

No excessive microbiological activity, relative to other samples, was observed for the samples collected from the school. The chemical parameters of the isolated sample did not exceed the limits stated for drinking water (SABS, 1984; Kempster & Smith, 1985). However, a strong petroleum/oil-like smell was noticed in these samples. This smell probably originated from unconventional substances (oils and greases) used during the installation of the school plumbing system. These substances probably caused the *Daphnia* lethality as *Daphnia* are known to be a very sensitive organisms (Slabbert, 1991). The first sample was collected immediately after a school holidays during which water was not used. This could have resulted in a build-up of these

substances. The reduction in toxicity after re-sampling indicates a dilution or removal of the substances from the plumbing system.

All samples collected from apartment buildings and private houses showed a *Daphnia* lethality of less than 10% after 48 hours. Samples collected from a research facility showed a lethality of 30% or less after 24 hours and a lethality of 75% or less after 48 hours. Total chlorine levels in these water samples varied from between 0,01 and 0,2 mg/ℓ. As *Daphnia pulex* is known to be highly sensitive to chlorine the lethality observed might be due to the chlorine levels recorded (Slabbert, 1991).

Several samples, collected in the metropolitan area, were tested for the presence of potentially mutagenic activity (Ames test). Mutagenic activity could not be detected in any of the samples tested. Govender and co-workers (1990) found Mutation Ratio (MR) values to vary from between 1,3 and 6,3, depending on the *Salmonella* strain used in their study, in drinking water for the same metropolitan area. A MR value greater than 2 indicates that a drinking water sample is mutagenic. However, Govender and co-workers (1990) have used a different concentration method in which large volumes (200 ℓ) of potable water were concentrated using XAD-7 resin.

As most of the samples tested for toxicity, using *Daphnia* pulex, showed negative results, it was decided not to carry out the endotoxin test. In studies carried out by Burger and co-workers (1989) very low endotoxin concentrations (<70 EU/mℓ) were detected in drinking water of the same metropolitan area. Presently, there are no limits for endotoxin levels in potable water. The occurrence and health implications thereof are as yet unclear and a controversial subject.

4.7. **Different materials supporting microbiological growth.**

Results from the questionnaires sent out to different water suppliers, indicated the piping material most frequently used in the major cities in South Africa consisted of cast-iron, concrete, PVC, polyethylene, bitumen lined steel and asbestos. In the light of these data and after consulting with technical experts, it was decided to evaluate the following materials *i.e.*, stainless steel, galvanized steel, mild steel, copper and PVC in

this study. Other materials were not commercially available in the desired thickness (1 mm).

A Pedersen's device was installed in a relatively old building, building no. 1 (32 years old) and a relatively new building, building no. 2 (3 years old). This was undertaken to allow for a comparison of the effect of the "age " of the plumbing system on biofilm formation. The surfaces of the selected materials were exposed to municipal running drinking water for a period of 12 weeks. The location of the device in both buildings was similar to allow for as close as possible conditions of flow and retention of water. Samples were collected after 1, 2, 4 and 12 weeks. The results obtained are summarized in Figs. 14 to 18.

In order to study the change in biofilm composition with time, for each of the materials investigated, a total of 197 bacterial cultures were isolated and purified from biofilms grown on the materials tested (PVC, 46; stainless steel, 29; galvanized steel, 26; mild steel, 52; copper, 44). The methods used for the identification of the organisms have been described in paragraph 3.6. The results are summarized in Tables 5 and 6.

4.7.1. **Mild steel**

Heterotrophic plate counts indicate mild steel to be the best supporter of bacterial growth (Fig. 14). Members of the genus *Pseudomonas* were found to be the dominant organisms growing on this material for the period of the first two weeks after which the genus *Bacillus* became dominant. This was found for both buildings no. 1 and no. 2 (Tables 5 & 6). Other organisms isolated were predominantly Gram-positive cocci, viz. *Staphylococcus, Deinococcus,* and *Micrococcus.* Members of the genus *Acinetobacter* (Gram-negative organisms) were isolated from building no. 1 after one week (Table 1).

4.7.2. **Stainless steel**

Relatively high bacterial counts were found on stainless steel (Fig. 17). The genus *Bacillus* was found to be the dominant organism in the biofilm formed on stainless

steel throughout the study period for building no. 1. However, in building no. 2 *Pseudomonas* was found to be the dominant organism after the first week, but was replaced from the second week on by *Bacillus*, similar to the finding for building no. 1 (Table 6). Other organisms (*Serratia, Alcaligenes, Flavobacterium, Serratia* and *Pseudomonas)* were isolated only from building no. 2.

4.7.3. **Galvanized steel**

Members of the genus *Bacillus* were found to be the dominant population on galvanized steel in building no. 1. Other organisms isolated were members of the genera *Pseudomonas, Staphylococcus* and *Micrococcus*. Sulphate-reducing bacteria were isolated from galvanized steel after 12 weeks. From the results (Table 5), it appeared as if members of the genus *Flavobacterium* were intermediately the dominant population (Week 1 & 4). No sulphate reducing bacteria were isolated from any of the other materials tested in both buildings. However, after 12 weeks, the genus *Bacillus* was found to be the dominant population. Other organisms isolated were *Pseudomonas* and *Micrococcus.*

4.7.4. **Copper**

Copper was found to be the best inhibitor of bacterial growth (Fig. 16). The dominant population found on copper in building no. 1 was identified as the genus *Bacillus.* Members of the genus *Pseudomonas* were found to be the dominant group on copper in building no. 2 (Table 6). Other organisms isolated were *Bacillus, Staphylococcus, Aeromonas* (building no. 1) and *Micrococcus* (building no. 2).

4.7.5. **PVC**

In comparison with galvanized steel and copper, relatively high bacterial counts were found on PVC (Fig. 18). The genus *Pseudomonas* was found to be the dominant population in building no. 2 (Table 6). In building no. 1, the dominant population was found to be of the genus *Achromobacter* (week 1) and *Pseudomonas* (week 2) (Table

5). However, after the fourth week, the genus *Bacillus* became the dominant population. Other organisms isolated were *Acinetobacter* (building no. 1) and *Staphylococcus* (building no. 2) and *Micrococcus* (building no.s 1 & 2).

4.7.6. **Comparison with other findings**

The results of this study are compare well with those of Gambassini and co-workers (1990). In another study carried out by Schoenen (1989) members of the genera *Pseudomonas* and *Flavobacterium* were the dominant organisms growing on the surface of different materials. *Alcaligenes* spp. and *Acinetobacter* spp. were frequently found and members of the genera *Bacillus, Micrococcus,* and *Planococcus* were only found sporadically. However, this is in contrast with the results found in this study and the study carried out by Gambassini and co-workers (1990). The constant and repeated isolation of the genus *Bacillus* can be attributed to its resistance to the action of chlorine.

4.8. **A comparison between the DAPI method and heterotrophic plate count method for counting planktonic cells**

Heterotrophic plate counts are mostly applicable to chemoheterotrophic bacteria (Van der Kooij *et al.* 1982). Chemoheterotrophic bacteria normally contribute to colony counts in distribution system. This group of bacteria includes opportunistic pathogens and other health-related bacteria (Geldreich *et al.*, 1972). Chemolitotrophic and chemoorganotrophic bacteria were also found to be present in the distribution system. Their absence in the bacterial populations found in this study might be due to the way water samples were enriched, rather than to an absence of these organisms from the reticulation system. This might explain the relatively higher DAPI counts (Fig. 14 - 18) which may be due to bacterial growth not being able to grow under the enrichment methods used in this study. All coupons showed overgrowth of bacteria after two weeks and dilutions were required prior to the counting of bacterial numbers using the DAPI technique. The variation in DAPI counts from one week to another (Tables 5 & 6) could be attributed to the deposition of debris which was found to interfere with the

counting procedure.

4.9. Corrosion tempos of different plumbing materials

Total corrosion tempos (CR) were determined for each of the materials tested. CR values were calculated after exposure of each of the coupons to municipal drinking water for a period of 12 weeks. Results are summarized in Fig. 19. Although relatively high counts were found on the stainless steel coupon (Fig. 17), it was found to be the most resistant material to corrosion. The highest corrosion tempo was found for mild steel, followed by galvanized steel and PVC. These results also indicate PVC to be less stable and inert than expected.

4.10. A comparison of the water quality of a relative old building and a relative new building

Results of the bacterial analyses of the different coupons clearly indicate a much higher count for building no. 1 than for building no. 2 (Fig. 14 - 18). Similar results were found for bacterial counts in the water samples collected (Fig. 20 - 24). Since the source and flow rate of the water were the same, it can be concluded that the "age" of the plumbing system plays an important role in the rate of biofilm formation, and indirectly influences the water quality. The isolation of faecal coliforms during the first phase of this study supports this conclusion. Higher CR values were also found for building no. 1 (Fig. 19).

5. CONCLUSIONS

- Although drinking water is treated using accepted and conventional treatment processes, taste and/or odour problems due to microbiological regrowth and aftergrowth occasionally do exist in the major centres in South Africa according to reports by water suppliers.

- Bacterial aftergrowth was found to occur in 1 of South Africa's major metropolitan areas but, did not affect the general potable water quality to a large extent. No specific bacterial pathogens, with the exception of *Legionella* bacteria, were isolated.

- Reservoirs on top of apartment buildings, where low levels of maintenance are practised, may pose a health risk to the inhabitants.

- The age of a building and its plumbing system plays an important role in the water quality. Much higher bacterial counts were found in water samples as well as biofilm samples collected from older buildings.

- The bacterial population of biofilms were found to concur with the population found in water samples.

- Mild steel was found to be the best supporter of bacterial growth in water distribution systems and copper the best inhibitor.

- Higher corrosion tempos are found in older buildings than those found in relatively new buildings.

References

Ames, B.C., McCann, J. and Yamasaki, E. 1975 Methods for detecting carcinogens and mutagens with the *Salmonella*/Mammalian-microsome mutagenicity test. *Mutations Research*, **31**, 347-364.

Aoyama, K. 1990 Studies on the earthy-musty odours in natural water (IV). Mechanism of earthy-musty odour production of actinomycetes. *Journal of Applied Bacteriology*, **68**, 405-410.

APHA, AWWA, WPCF 1985 *Standard Methods for the Examination of Water and Wastewater*. 6th edn. ed. Greenberg, A.E., Trussel, R.R. and Clesceri, L.S., Washington DC: American Public Health Association.

Berg, J.D., Martin, A. and Roberts, P.V. 1981 Growth of disinfection resistant bacteria

and simulation of natural aquatic environments in chemostat. In *Water Chlorination: Environmental Impact and Health Effects*, vol. 5, pp.219-243. Ann Arbor: Ann Arbor Science.

Bondonno, A. 1990 Materials Science and Technology, CSIR, Personal Communication.

Burger, J.S., Grabow, W.O.K. and Kfir, R. 1989 Detection of endotoxins in reclaimed and conventionally treated drinking water. *Water Research*, **23**, 733-738.

Doetsch, R.N. 1981 Determinative methods of light microscopy. In Manual of Methods for General Bacteriology ed. Gerhardt, P., Murray, R.G.E., Costilow, R.N., Nester, E.W., Wood, W.A., Krieg, N.R. & Phillips, G.B. pp. 21-33 American Society for Microbiology, Washington, DC 20006.

EPA, 1985 *Methods for measuring the acute toxicity of effluents to freshwater and marine organisms*. EPA/600/485/013, Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio.

Gambassini, L., Sacco, C., Lanciotti, E., Burrini, D. and Griffini, O. 1990 Microbial quality of the water in the distribution system of Florence. *Aqua*, **39**, 258-264.

Geldreich, E.E. *et al.* 1972 The necessity of controlling bacterial populations in potable water: Community water supply. *Journal of American Water Works Association*, **64**, 596.

Gerber, N.N. 1983 Volatile substances from actinomycetes; their role in odour pollution of water. *Water Science and Technology*, **15**, 115-125.

Gerhardt, P., Murray, R.G.E., Costilow, R.N., Nester, E.W., Wood, W.A., Krieg, N.R. & Phillips, G.B. 1981 Manual of Methods for General Bacteriology, American Society for Microbiology, Washington, DC 20006.

Grabow, W.O.K. 1989 Department of Medical Virology, University of Pretoria, Personal Communication.

Grabow, N.A., Kfir, R. and Grabow, W.O.K. 1991 A most probable number method for the enumeration of *Legionella* bacteria in water. *Water Science and Technology*, **24**, 143-147.

Gregerson, T. 1978 A rapid method for distinction of Gram-negative from Gram-positive bacteria. *European Journal of Applied Microbiology and Biotechnology*, **5**, 123-127.

Govender, V., Meintjies, E. and Hassett, D.W. 1990 Survey of mutagens in South

African drinking waters. Contract Project for the Department of National Health and Population Development. August 1990, Health Programme, Division of Water Technology, CSIR.

Havelaar, A. H., During, M. and Delfgou-Van Asch, E.H.M. 1985 Comparative study of membrane filtration and enrichment media for the isolation and enumeration of *Pseudomonas aeruginosa* from sewage, surface water and swimming pools. *Canadian Journal of Microbiology*, **31**, 686-692.

Havelaar, A.H., During, M. and Versteegh, J.F.M. 1987 Ampicillin-dextrin agar medium for the enumeration of *Aeromonas* species in water by membrane filtration. *Journal of Applied Microbiology*, **62**, 279-287.

Hinzelin, F. and Block, J.C. 1985 Yeasts and filamentous fungi in drinking water. *Environmental Technology Letter*, **6**, 101-106.

Holdeman, L.V., Cato, E.P. & Moore, W.E.C. 1977 Anaerobe Laboratory Manual 4th edn. Virginia Polytechnic Institute. Blacksburg: Virginia.

Kempster, P.L. and Smith, R. 1985 *Proposed aesthetic/physical and inorganic drinking-water criteria for the Republic of South Africa.* CSIR Report no. 628, National Institute of Water Research, Council for Scientific and Industrial Research.

Kfir, R., Grabow, W.O.K. and Hilner, C.A. 1982 Ames *Salmonella* assay in water: Dichlorination extracts versus preparation of growth media with test samples. *Bulletin for Environmental Toxicology*, **23**, 641-646.

Kfir, R. 1989 Recommended health guidelines for drinking water. *Community Health in South Africa*, **4**, 26-29.

Krieg, N.R. and Holt, J.G. 1984 *Bergey's Manual of Systematic Bacteriology.* Vol. 1. London: Williams & Wilkins.

Langelier, W.F. 1936 The analytical control of anticorrosion water treatment. *Journal of American Water Works Association*, **28**, 1500.

LeChevallier, M.W., Cameron, S.C. and McFeters, G.A. 1982 New medium for improved recovery of coliform bacteria from drinking water. *Applied and Environmental Microbiology*, **45**, 484-492.

LeChevallier, M.W. and McFeters, G.A. 1985 Enumerating injured coliforms in drinking water. *Journal of American Water Works Association*, **77**, 81-87.

Marcus, L., Vismer, H.F., Gove, E.W., Meewes, P.J. and Van der Hoven, H.J. 1991 Mycotic keratitis caused by *Curvularia brachyspora*: A report of the first case. In *Proceedings of the 31st Annual Congress of the Federation of South African*

Societies of Pathology, Overvaal Resort, Warmbaths, 30 June - 3 July 1991.

McFeters, G.A., Kippen, J.S. and LeChevallier, M.W. 1986 Injured coliforms in drinking water. *Applied and Environmental Microbiology*, **51**, 1.

McFeters, G.A. and Singh, A. 1988 The virulence of waterborne pathogenic bacteria following drinking water injury. In *Proceedings of the Water Quality Technology Conference*, St. Louis, Missouri, November 13-17, 1988, pp. 549-560.

Mittelman, M.W. and Geesey, G.G. 1987 *Biological Fouling of Industrial Water Systems: A Problem Solving Approach*. Water Micro Associates, San Diego, California.

Pedersen, K. 1982 Method for studying microbial biofilms in flowing water systems. *Applied and Environmental Microbiology*, **43**, 6-13.

Pfennig, N. & Wagener, S. 1986 An improved method of preparing wet mounts for photomicrographs of microorganisms. *Journal of Microbiological Methods*, **4**, 303-306.

Reasoner, D.J. 1988 Drinking water microbiology research in the United States: An overview of the past decade. *Water Science and Technology*, **20**, 101-107.

Rosenzweig, W.D. and Pipes, W.O. 1988 Fungi from potable water: Interaction with chlorine and engineering effects. *Water Science and Technology*, **20**, 153-159.

SABS 1984 *Spesifikasie vir Water vir Huishoudelike Gebruik*, Suid Afrikaanse Standaard, SABS 241-1984, Gr 7, Tweede hersiening, Die Raad van die Suid-Afrikaanse Buro vir Standaarde.

Schoenen, D. 1989 Influence of materials on the microbiological colonization of drinking water. *Aqua*, **38**, 101-113.

Skadsen, J. 1988 Bacterial regrowth in a distribution system and comparison of m-Endo broth and MT7 agar for coliform recovery. In *Proceedings of the Water Quality Technology Conference*, St. Louis, Missouri, November 13-17, 1988, pp.527-547.

Slabbert, L. 1991 Health Programme, Watertek, CSIR, Personal Communication.

Starr, M.P., Stolp, H., Trüper, H.G., Barlows, A. and Schlegel, H.G. 1981 *The Prokaryotes*. Vol. 1 & 2, New York: Springer-Verlag.

STSC 1989 Statgraphics 4.0. Statistical Graphics Corporation, STSC Inc., Maryland, USA.

Van der Kooij, D. 1982 The determination of the aftergrowth possibilities of bacteria in drinking water. In *Proceedings of the Development of Methods For Determining Water Quality Seminar*, Rijswijk, The Netherlands, pp. 6-8.

Van der Kooij, D., Visser, A. and Hijnen, W.A.M. (1982). Determining the concentration of easily assimilated organic carbon in drinking water. *Journal of the American Water Works Association*, **74**, 540-545.

Victoreen, H.T. 1974 Control of water quality in transmission and distribution mains. *Journal of American Water Works Association*, **66**, 369.

Von Holy, A. 1987 New approach to microbiologically induced corrosion. *Water, Sewage & Effluent*, Sept. 1987, 39-44.

Waksman, S.A. 1961 *The Actinomycetes*. New York: The Ronald Press Company.

Williams, S.T., Sharpe, M.E. and Holt, H.J.G. 1989 *Bergey's Manual of Systematic Bacteriology*, vol. 4. London: Williams & Wilkins.

Wilson, J.W. and Plunkett, O.A. 1967 *The fungous diseases of man*. Los Angeles: University of California Press,

Wolfaart, G., Smith, A., Smith, F. and Cloete, T.E. 1990 Techniques for biofouling monitoring during alkaline paper manufacturing. In *Proceedings of the Fifth South African Pulp and Paper Technical Conference*, Johannesburg, June 1990.

APPENDIX A

Questionnaire sent out to municipalities and waterboards

DIVISION OF WATER TECHNOLOGY

HEALTH PROGRAMME

QUESTIONNAIRE

Name and address of Institution: _____

Tel: _____ _____

_____

1. Volume of water supplied per year: _____
2. Estimate total length of the pipes in km: _____
3. What is the water source if there is more than one. Please specify the main ones: _____
4. From what materials are the pipes constructed?: _____
5. If the water is treated, what are the stages?: _____
6. The water at the distribution system is maintained under _____ mg/ℓ of residual chlorine.
7. Average number of coliform bacteria in the raw water is: _____
8. Average number of total bacterial count at the distribution system is _____ organisms/mℓ.
9. The chemical water quality at the distribution system (if it is evenly distributed) for Ca_____mg/ℓ; Mg_____mg/ℓ; P_____mg/ℓ; NO_3_____mg/ℓ; Temp_____; pH_____; Permanganate demand_____mg/ℓ.

   If it is different in a few areas please indicate an additional sampling point, and provide the data:
   Ca_____mg/ℓ; Mg_____mg/ℓ; P_____mg/ℓ; NO_3 _____mg/ℓ; Temp_____; pH_____; Permanganate demand_____mg/ℓ.
10. Is there any bacterial growth problem in the distribution system? **YES/NO**. If the answer is **YES** please continue, if the answer is **NO** then please carry on as from No. 13.
11. How was it discovered - physical, chemical, flow measurements, or other means?: _____
12. Was there any difference in bacterial growth appearance at the various diameters of the pipes?: _____

13. Was there any difference in bacterial growth appearance at points close to the water treatment plant, or at points that are at the end of the distribution system?:

_____

14. Were any pathogenic bacteria isolated from **R**aw, **F**inished, **D**istribution water. Please indicate Y = yes; N = no, at the right place?:
R_____; F_____; D_____.

15. If known, what was the name of the bacteria and numbers isolated?:_____

_____

16. Was there at anytime a trail to get rid of the bacterial growth?:_____

_____

17. If yes - what steps were taken?: _____

_____

18. Did it help?_____, if **Yes**, for how long was it effective?:_____

_____

19. Were there at any times complaints from the public concerning objectionable taste or odour (or both) in the system?:_____

20. What type of treatment was introduced in order to overcome it?_____

_____

21 For how long was it useful?:_____

22. Did you have to repeat the treatment procedure?:_____

23. Was the cause of taste , odour (or both) identified?:_____

_____

24. If the answer to 22 is **YES**, would you mention the name of the organism responsible for it?:_____

Thank you for your patience and cooperation

In-house questionnaire

QUESTIONNAIRE

Serial No.	Suburb	Has the water occasionally had			When was it last noticed	Did you complain to the municipality	Did it help	Remarks
		Taste		Odour				
		By chlorine	Other					

Questnai/Kott

APPENDIX B

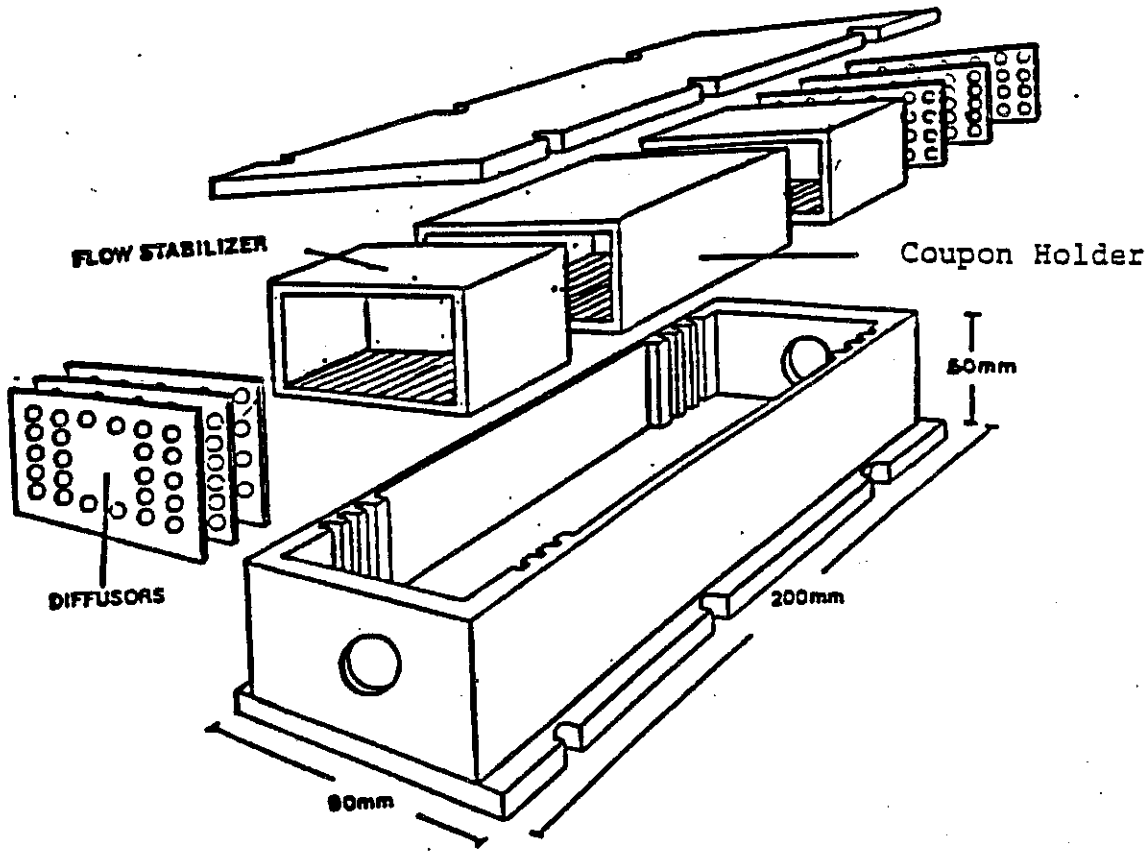

Fig. 1 A modified Pedersen device

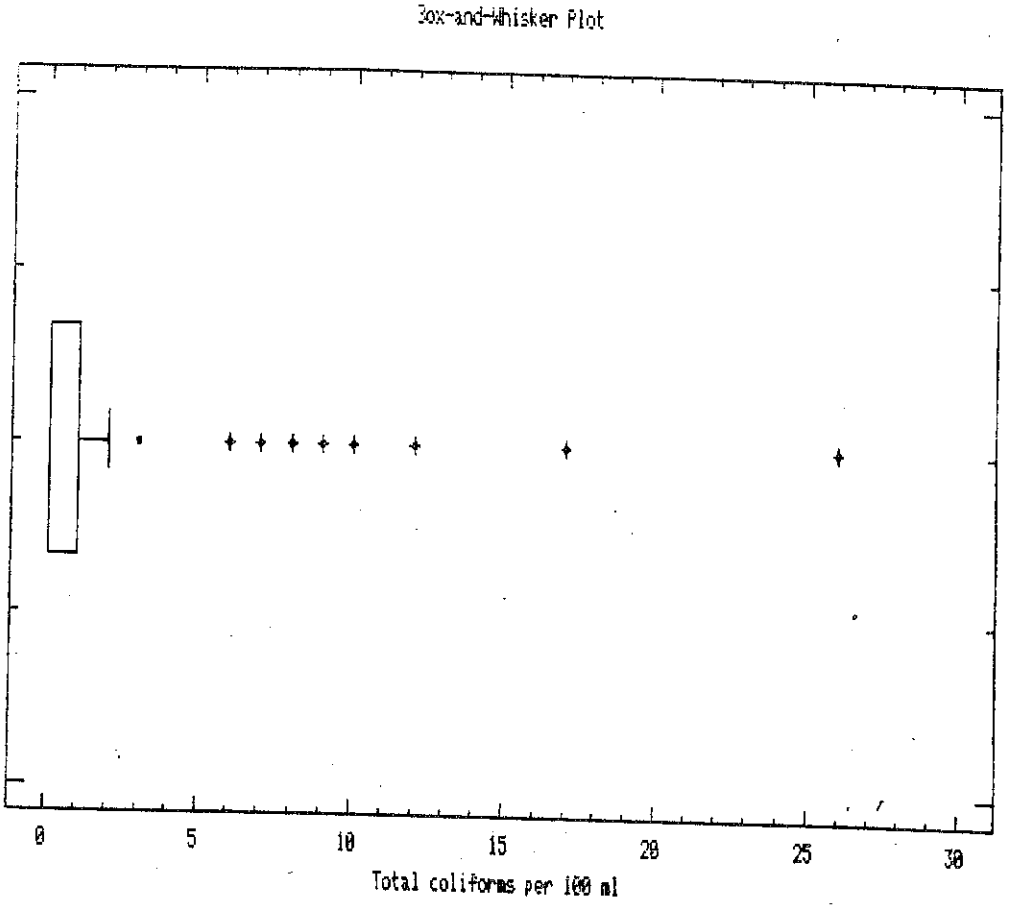

Fig. 2 Numbers of total coliforms counted in water samples over a 2 year period.

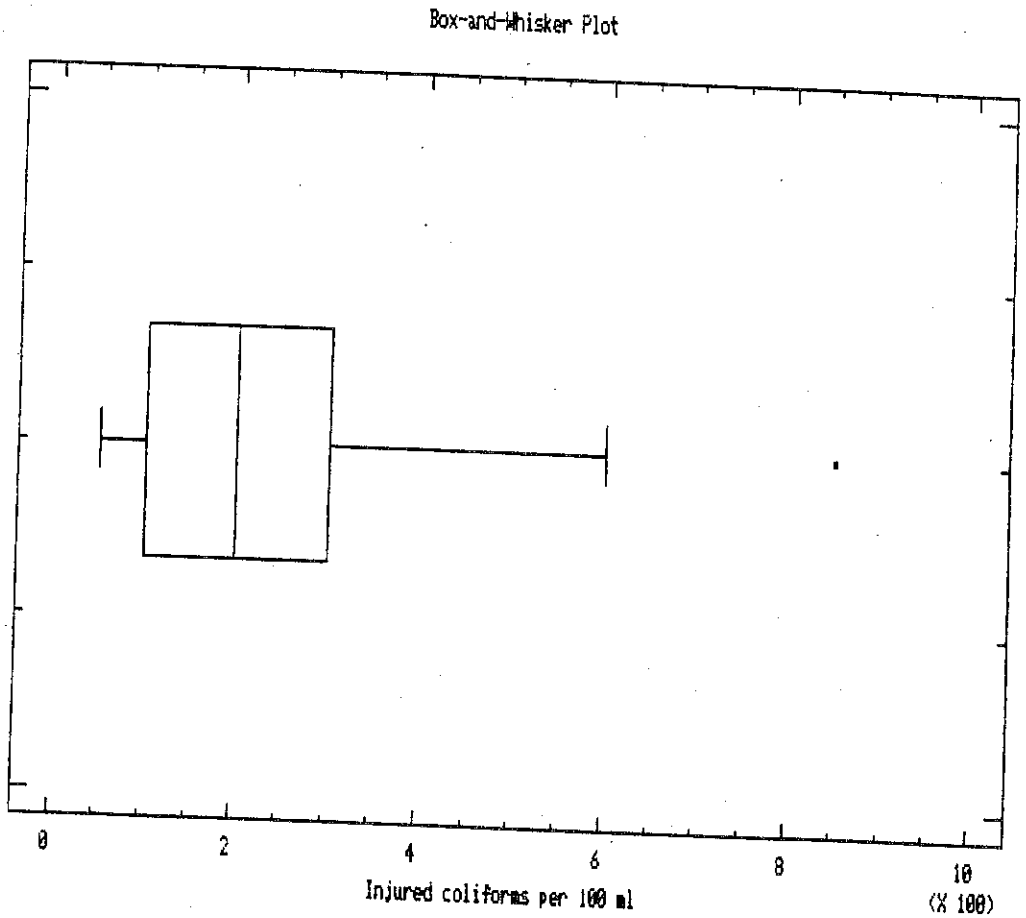

Fig. 3 Numbers of injured coliforms counted in water samples over a 2 year period.

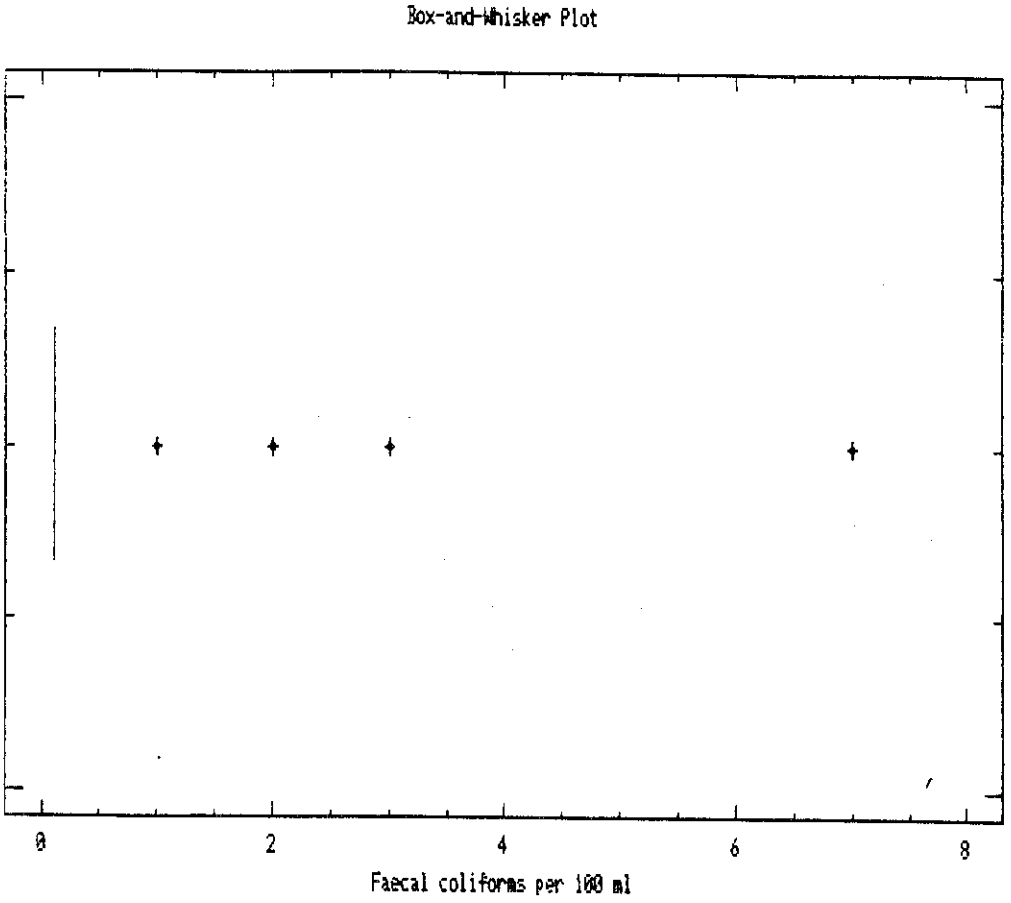

Fig. 4 Numbers of faecal coliforms counted in water samples over a 2 year period.

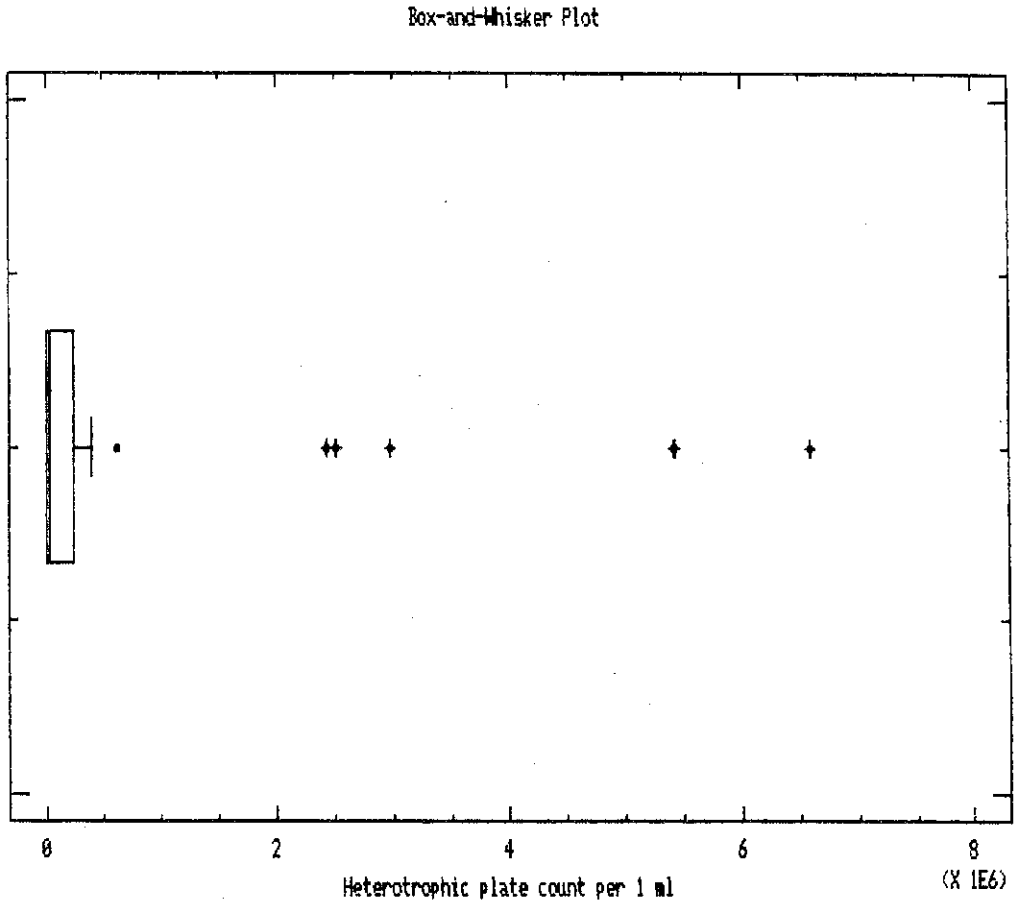

Fig. 5 Heterotrophic plate counts found in water samples over a 2 year period.

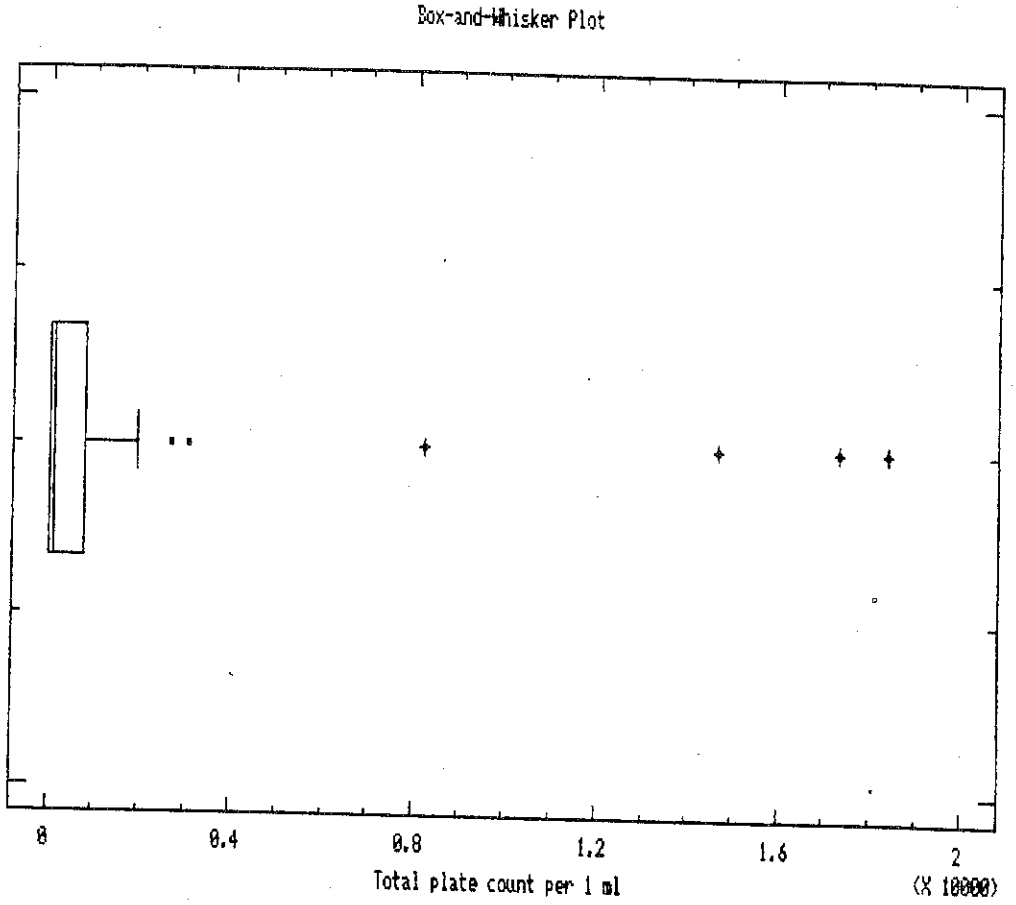

Fig. 6 Total plate counts found in water samples over a 2 year period.

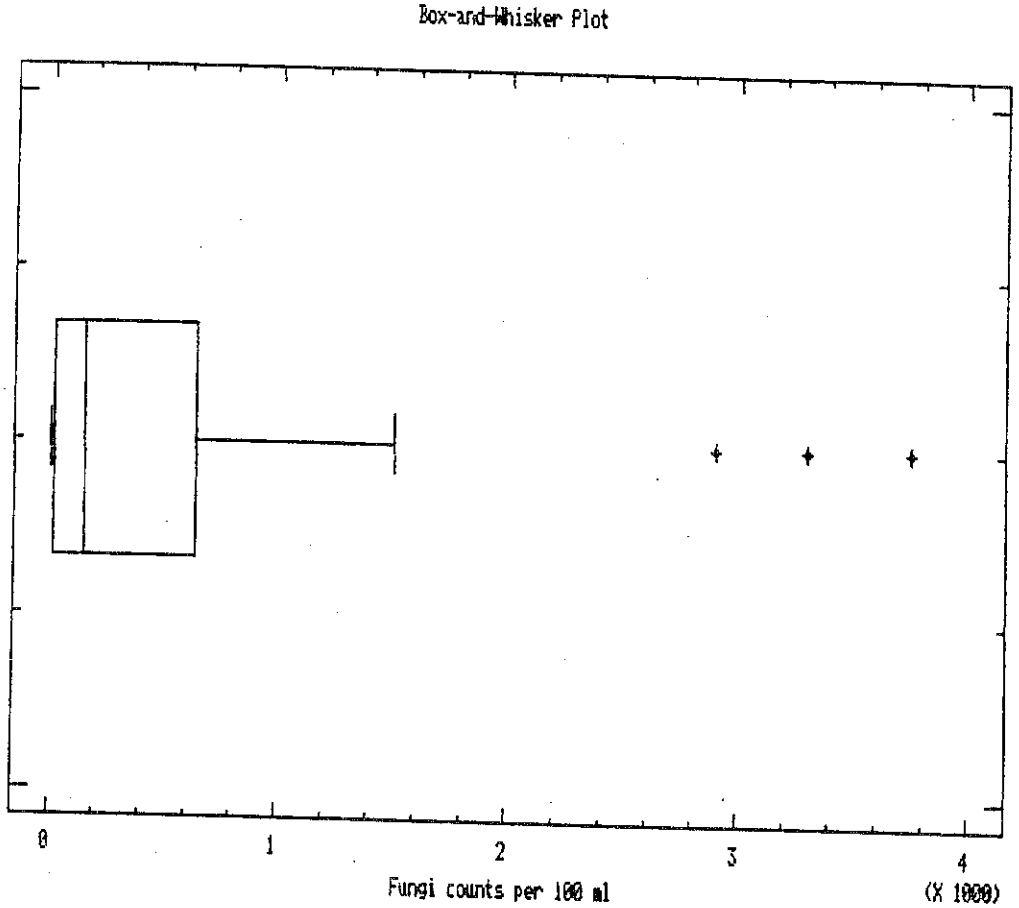

Fig. 7 Numbers of fungi counted in water samples over a 2 year period.

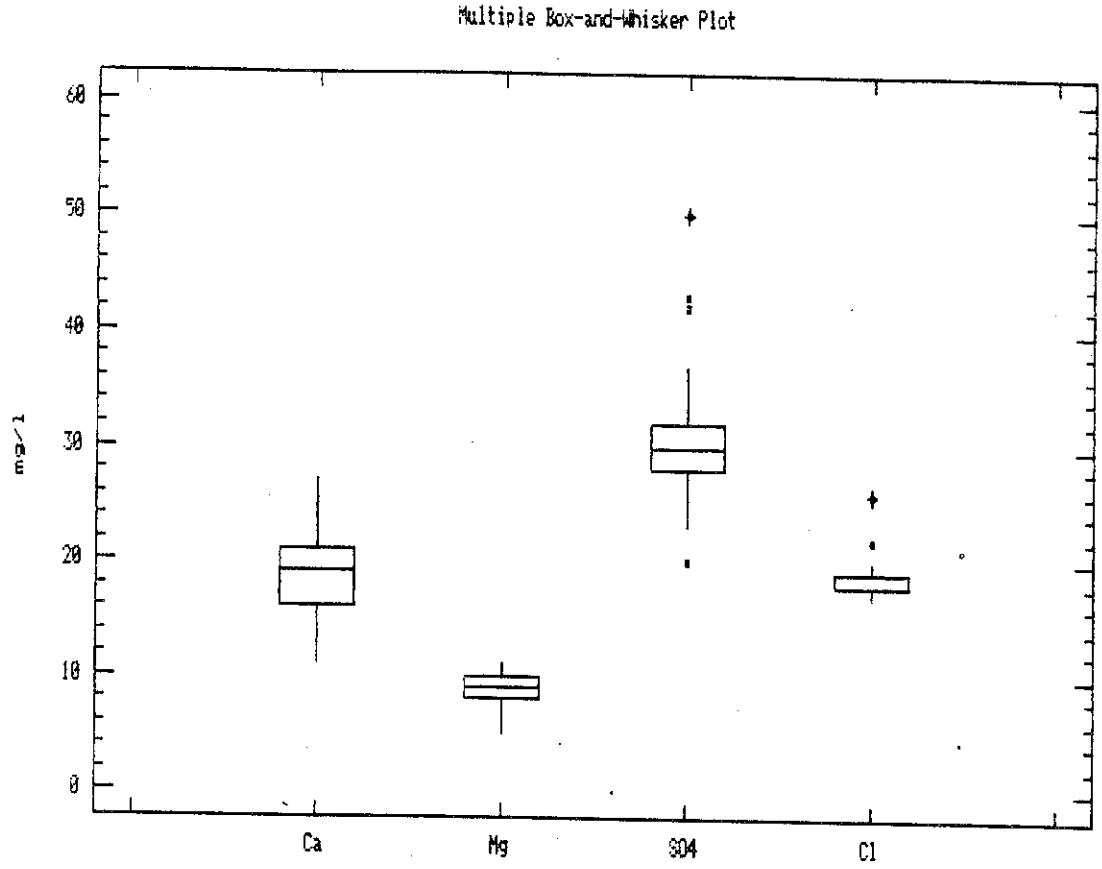

Fig. 8 A summary of the different inorganic salt concentrations found in water samples over a 2 year period.

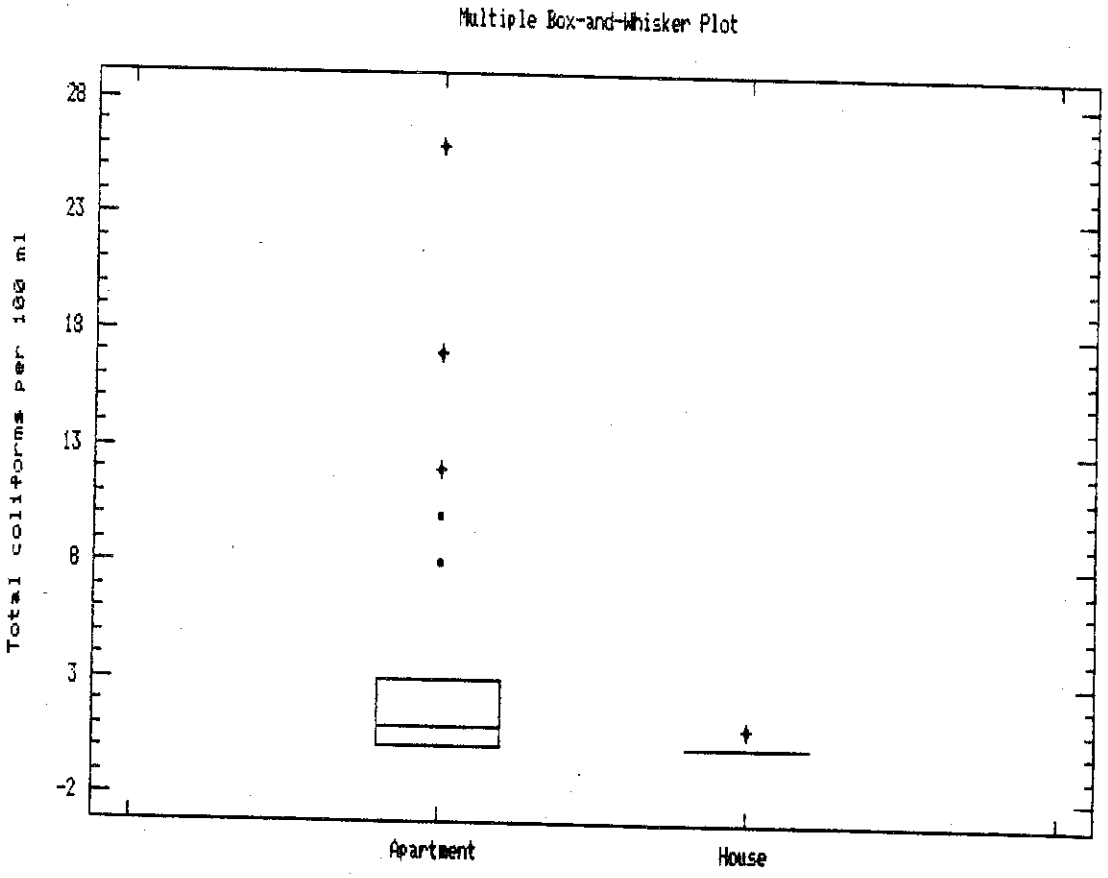

Fig. 9 A comparison between numbers of total coliforms detected in apartment buildings and private houses.

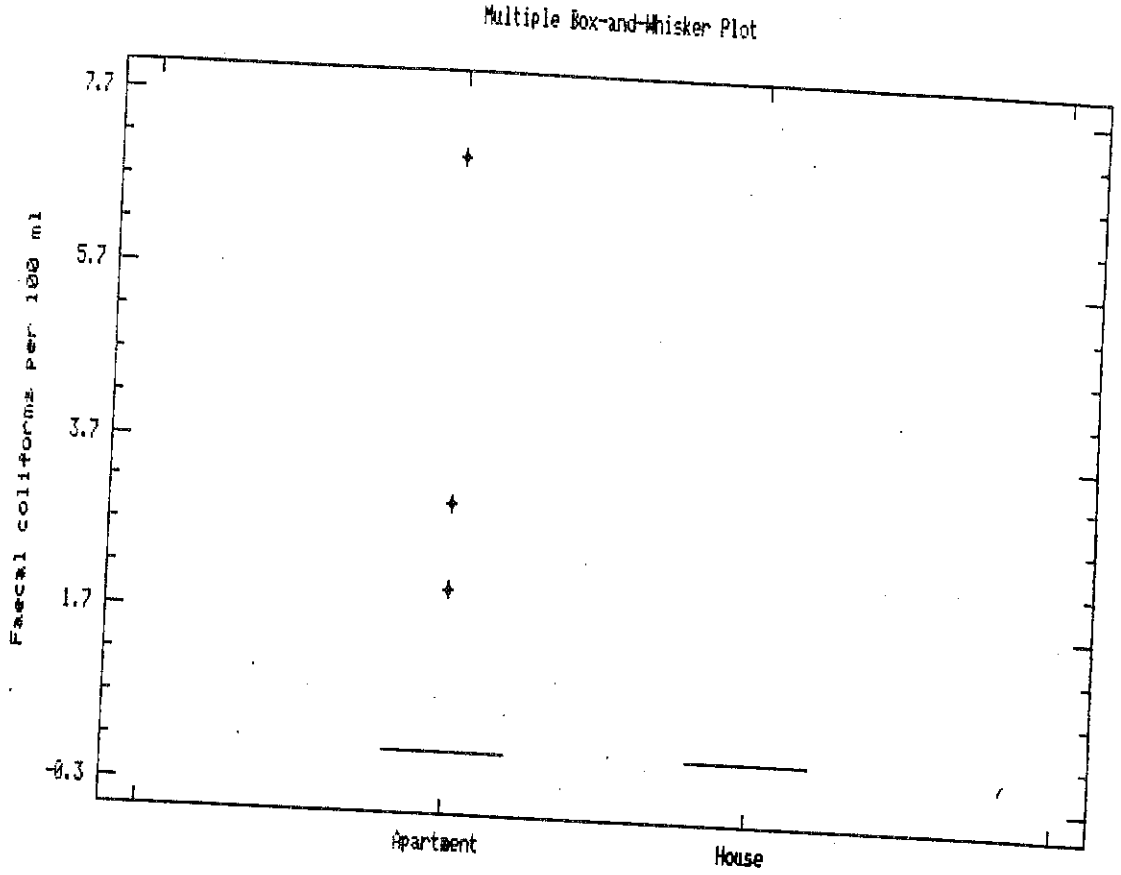

Fig. 10 A comparison between numbers of faecal coliforms detected in apartment buildings and private houses.

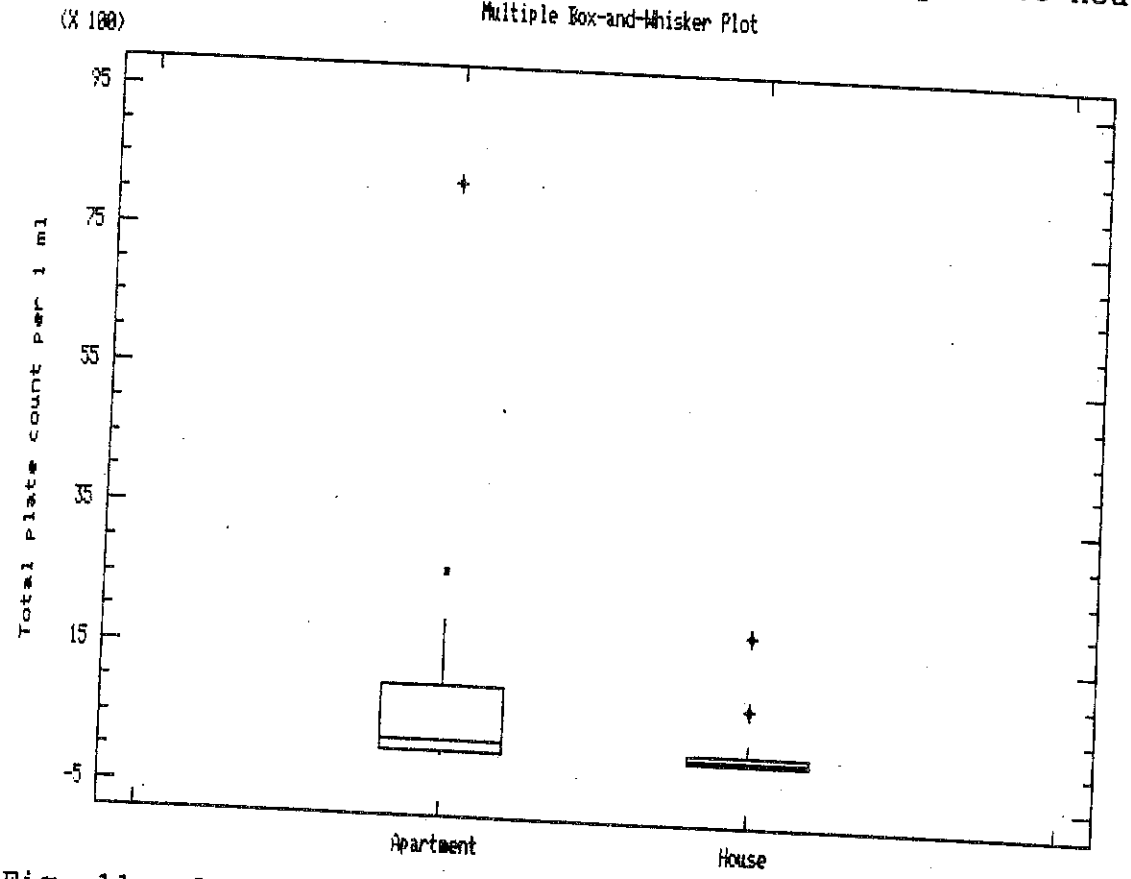

Fig. 11 A comparison between numbers of total plate counts detected in apartment buildings and private houses.

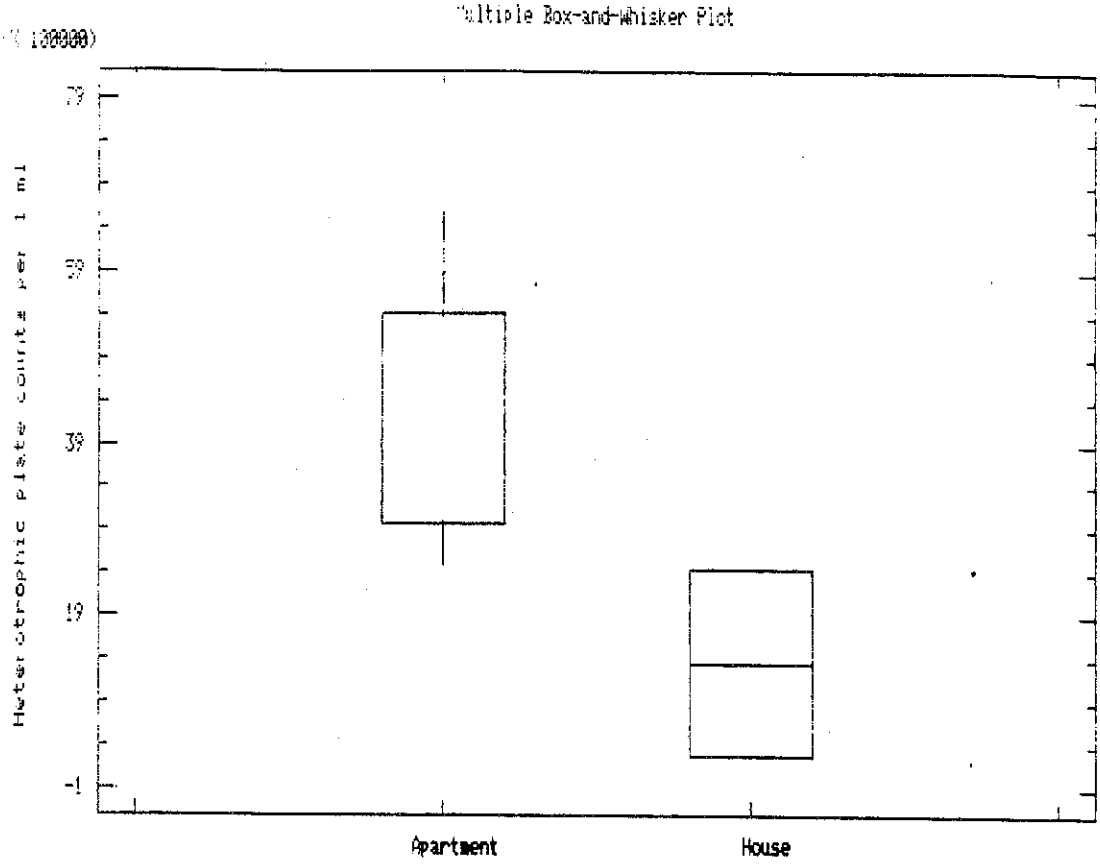

Fig. 12 A comparison between heterotrophic plate counts detected in apartment buildings and private houses.

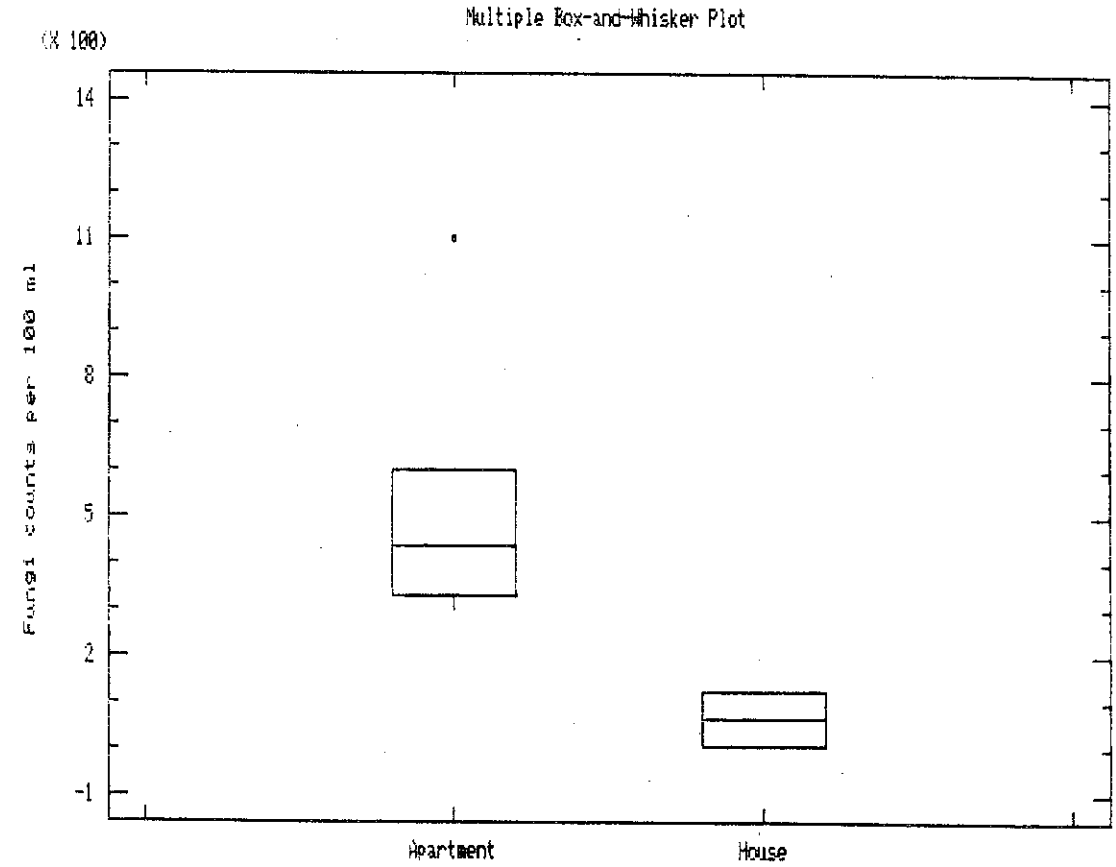

Fig. 13 A comparison between numbers of fungi detected in apartment buildings and private houses.

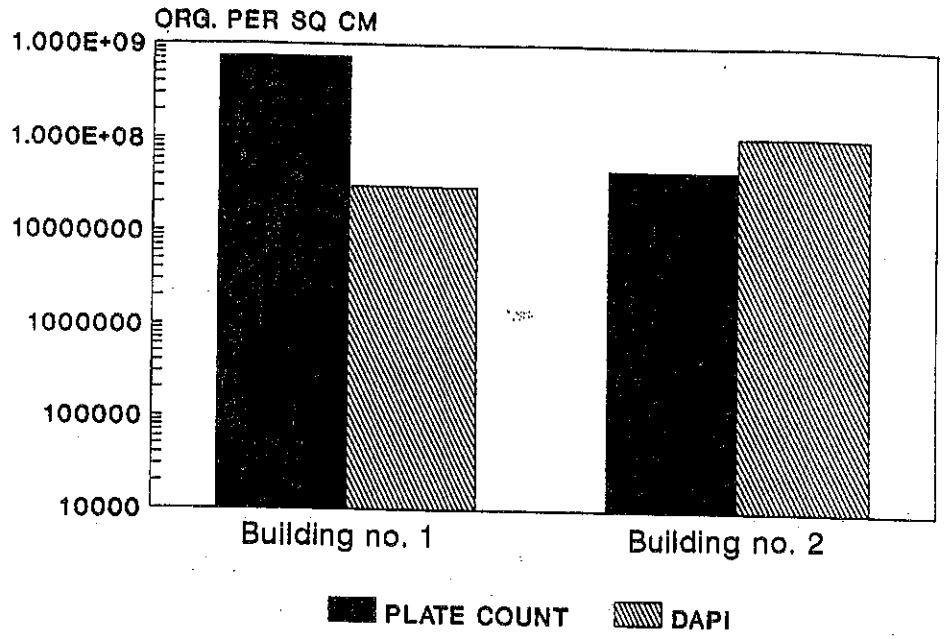

Fig. 14 Bacterial counts on mild steel after 12 weeks.

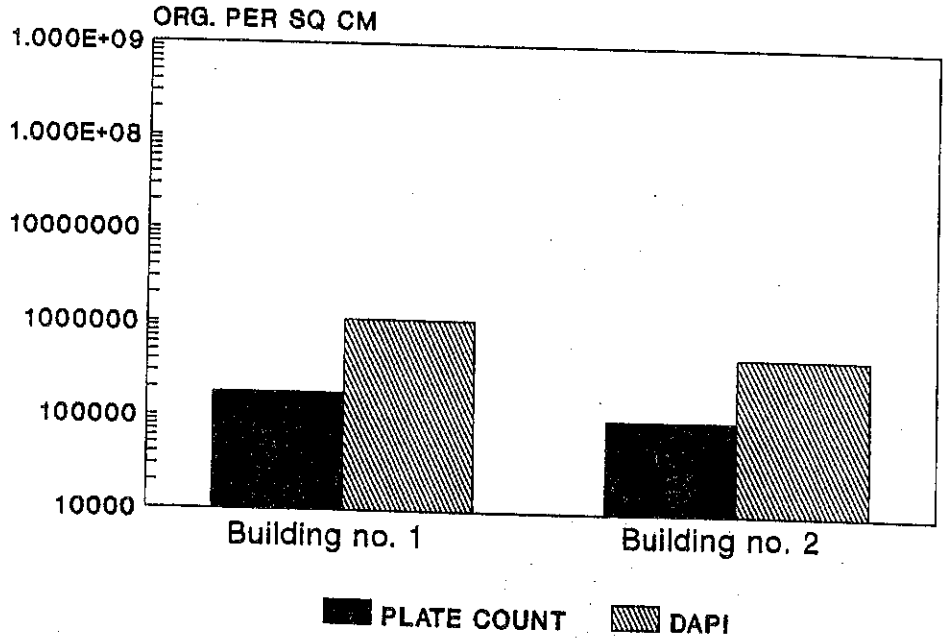

Fig. 15 Bacterial counts on galvanized steel after 12 weeks.

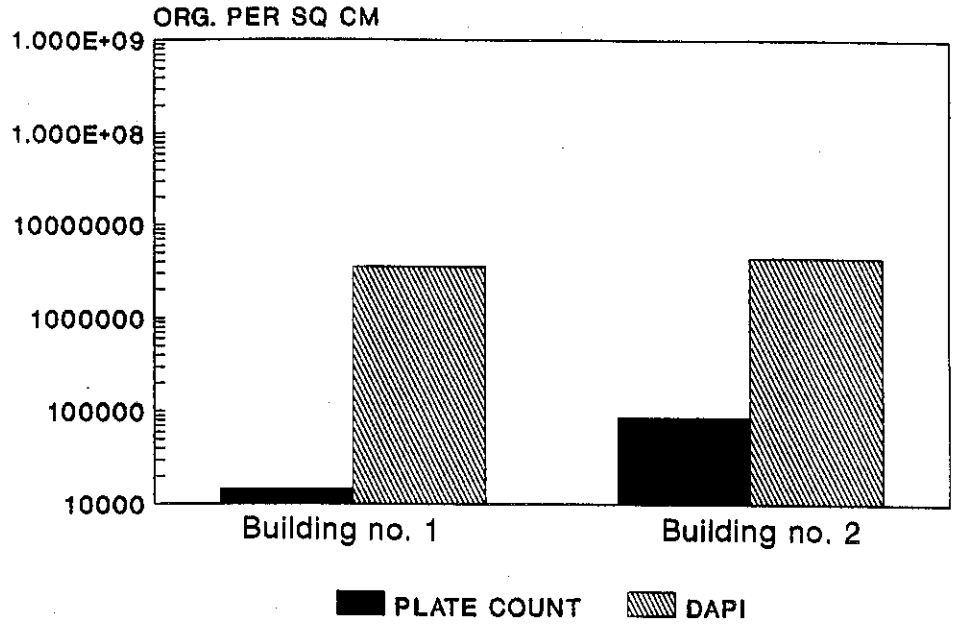

Fig. 16 Bacterial counts on copper after 12 weeks.

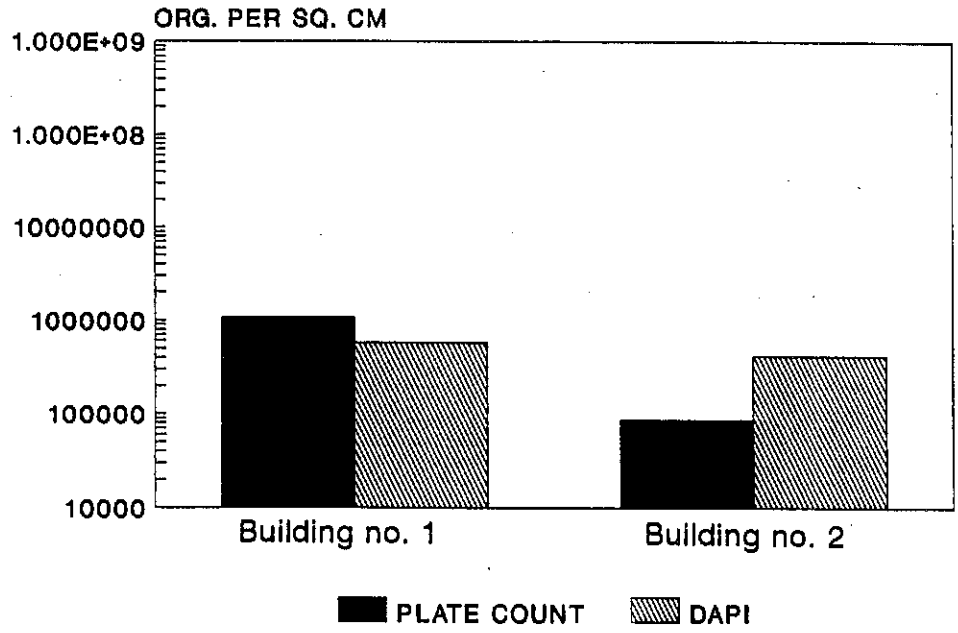

Fig. 17 Bacterial counts on stainless steel after 12 weeks.

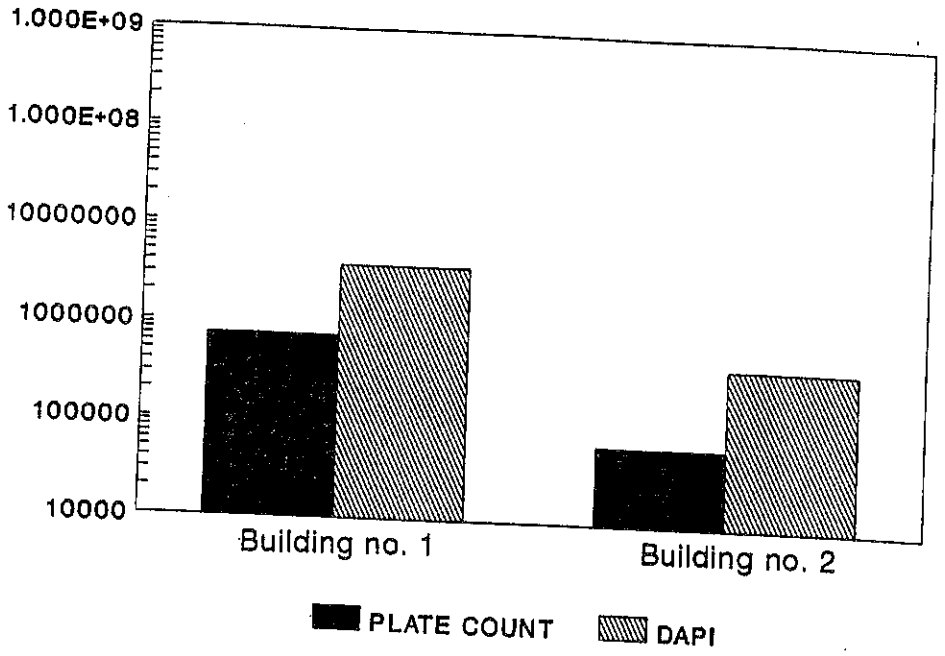

Fig. 18 Bacterial counts on PVC after 12 weeks.

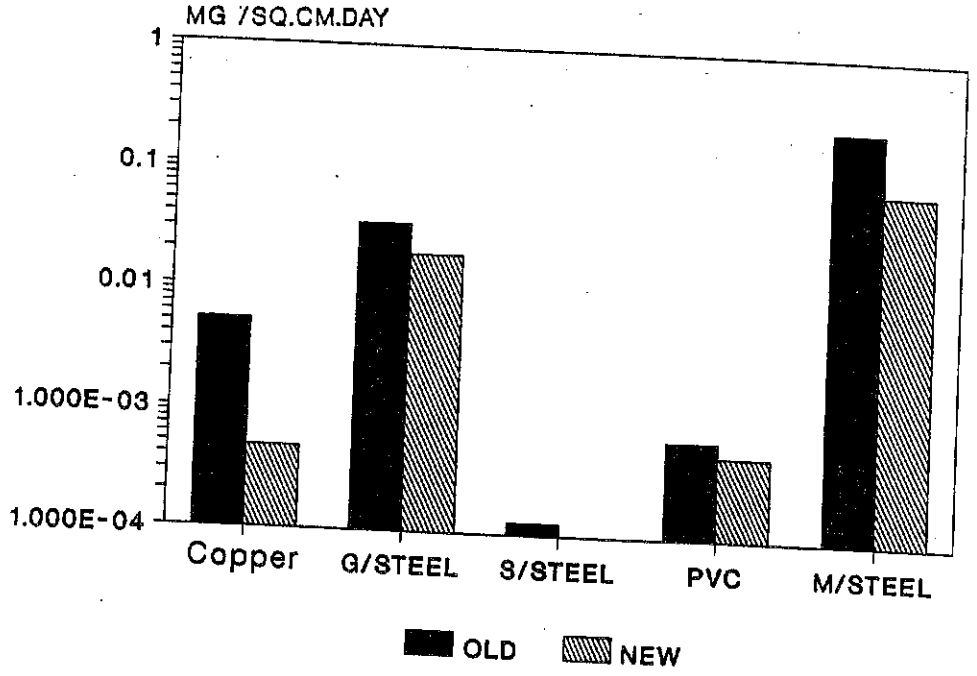

Fig. 19 Corrosion tempos for each material tested after 12 weeks.

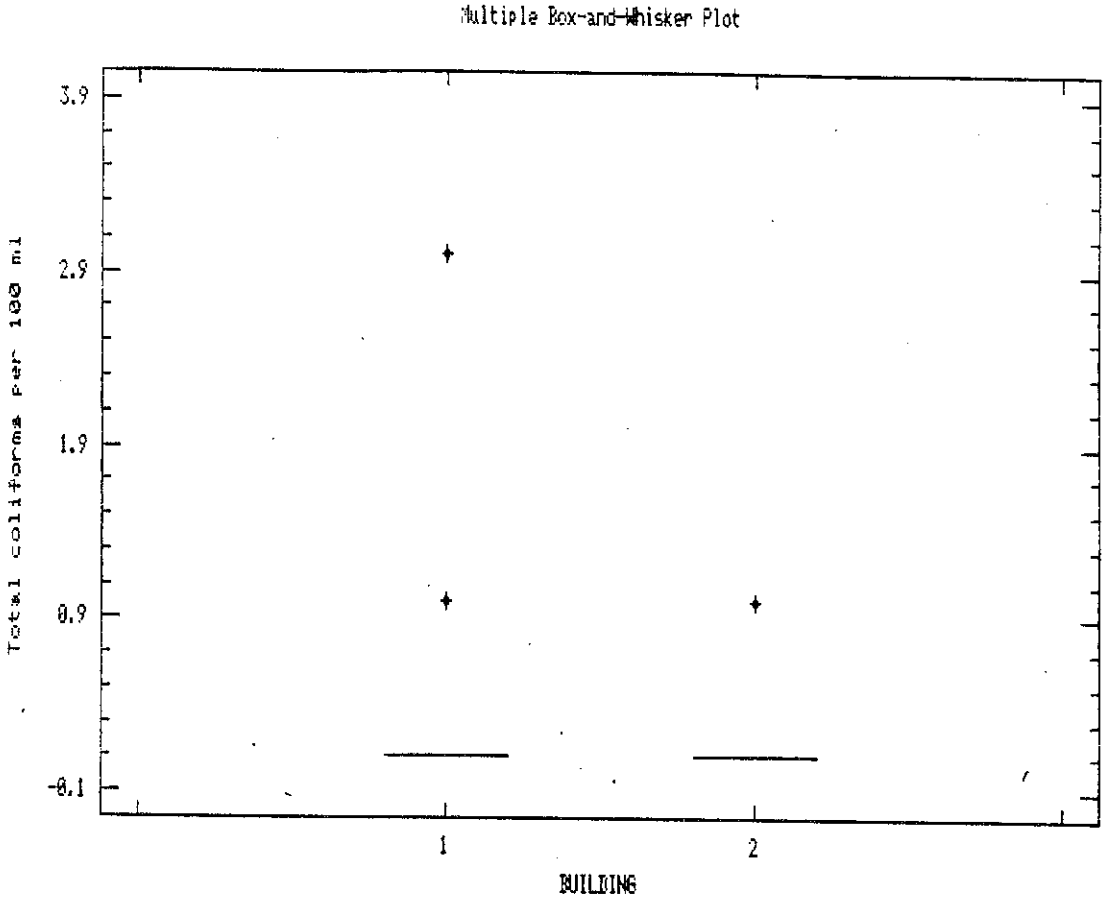

Fig. 20 A comparison between numbers of total coliforms detected in an old and new building.

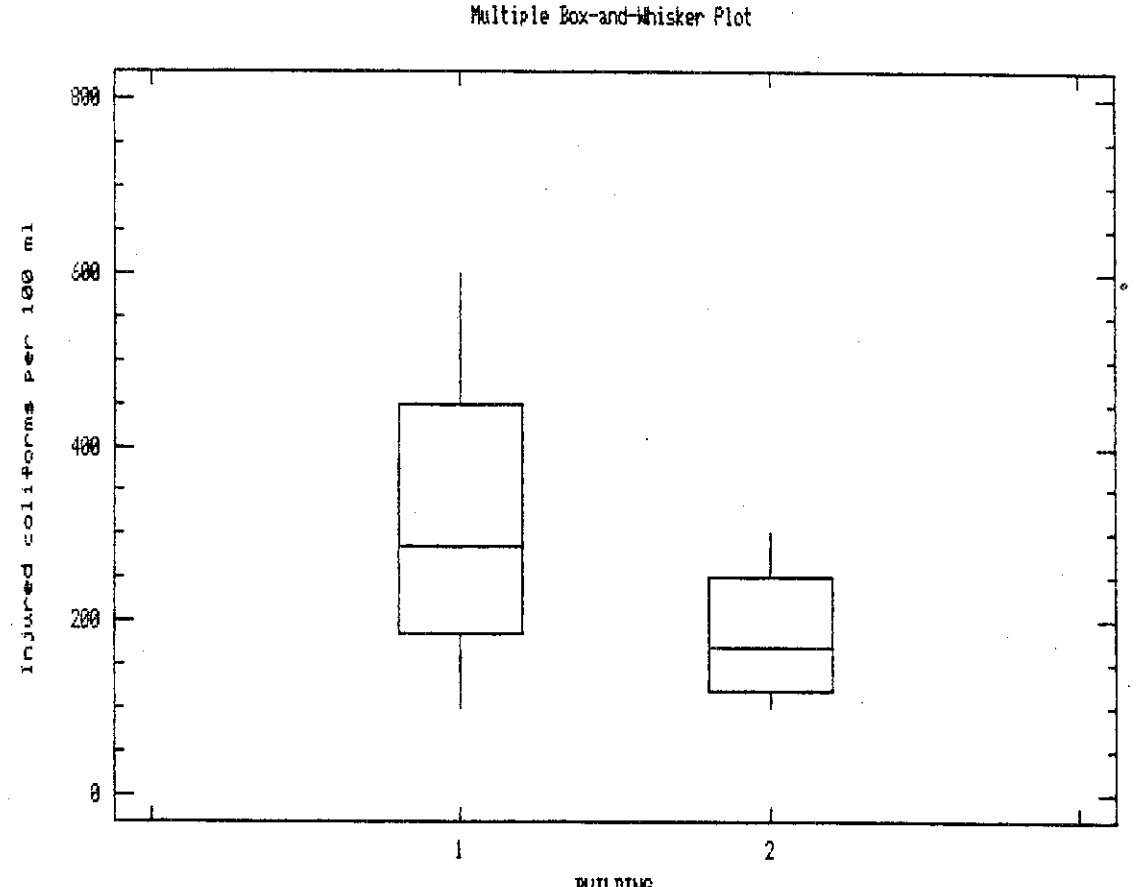

Fig. 21 A comparison between numbers of injured coliforms detected in an old and new building.

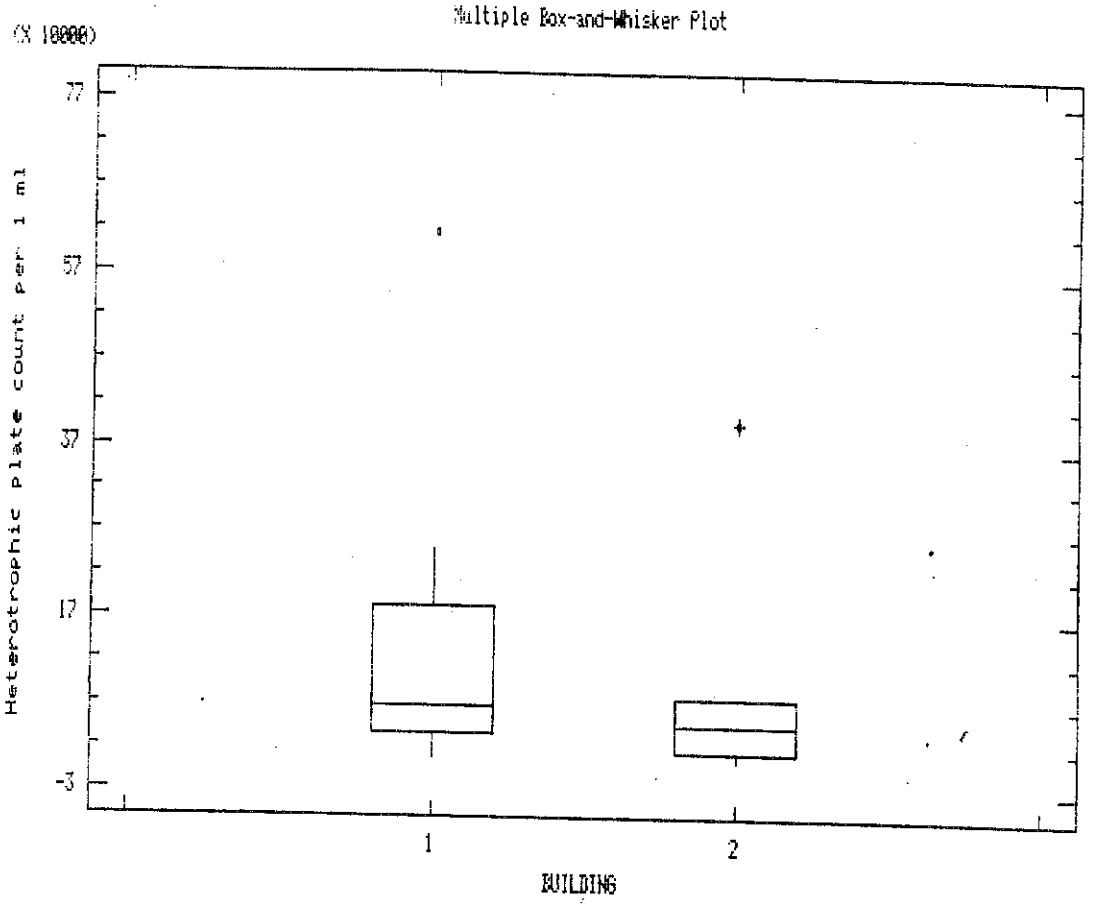

Fig. 22 A comparison between numbers of heterotrophic plate counts detected in an old and new building.

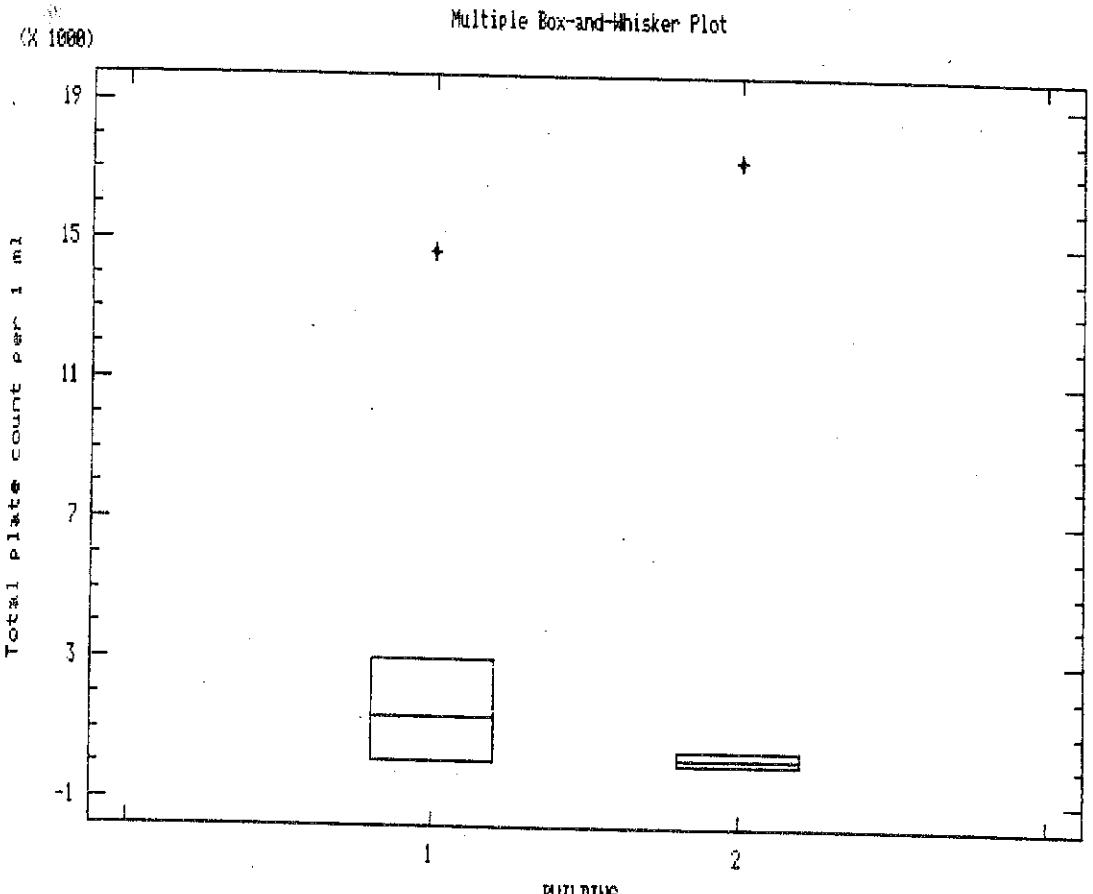

Fig. 23 A comparison between total plate counts detected in an old and new building.

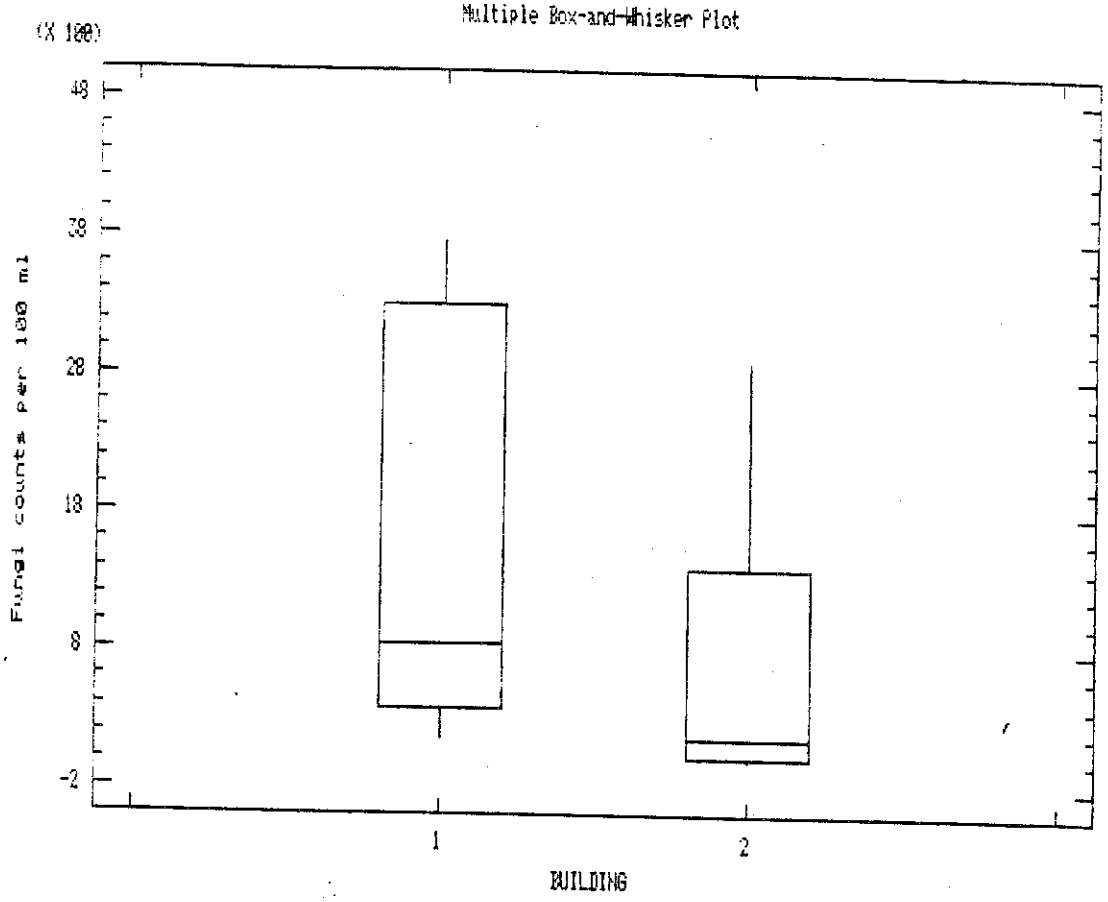

Fig. 24 A comparison between numbers of fungi detected in an old and new building.

APPENDIX C

Table 1. A summary of the microbiological results of samples collected from house no. 1 in a relatively old suburb.

Enrichment	Total coliforms (TC)	Faecal coliforms	*Aeromonas*	*Klebsiella*	*Pseudomonas*	*Legionella*	Total plate count*
WATER SAMPLES:							19 CFU/mℓ
Peptone broth	Growth TC negative	No growth	ND	Heavy growth *Klebsiella* neg.	No growth	Heavy growth *Legionella* neg.	
Nutrient broth	No growth	No growth	ND	No growth	No growth	Heavy growth *Legionella* neg.	ND
SCRAPING:							
Peptone broth	Heavy growth TC neg.	No growth	Heavy growth *Aeromonas* neg.	Heavy growth *Klebsiella* neg.	No growth	Heavy growth *Legionella* neg.	
Nutrient broth	Heavy growth TC neg.	No growth	Heavy growth *Aeromonas* neg.	No growth	No growth	Heavy growth *Legionella* neg.	

* - A total plate count was performed on the water sample only, before enrichment.
ND - Not done

Table 2. A summary of the microbiological results of samples collected from house no. 2 in a relatively old suburb.

Enrichment	Total coliforms (TC)	Faecal coliforms (FC)	*Aeromonas*	*Klebsiella*	*Pseudomonas*	*Legionella*	Total plate count*
WATER SAMPLES:							
Peptone broth	Growth TC negative	No growth	ND	Heavy growth *Klebsiella* neg.	No growth	Heavy growth *Legionella* neg.	117 CFU/mℓ
Nutrient broth	No growth	No growth	ND	No growth	No growth	Heavy growth *Legionella* neg.	
SCRAPING:							
Peptone broth	No growth	No growth	No growth	No growth	No growth		ND
Nutrient broth	TC pos. (102 CFU)	FC pos. (104 CFU)	Growth *Aeromonas* neg.	No growth	No growth	Heavy growth *Legionella* neg.	
						Heavy growth *Legionella* neg.	

* - A total plate count was performed on the water sample only, before enrichment.
ND - Not done

Table 3. A summary of the chemical/physical parameters of water samples collected in a major metropolitan area.

	DOC (mg/ℓ)	TOC (mg/ℓ)	AOC (µg/ℓ)	TEMP (°C)	pH	CONDUCTIVITY (mS/m)	HARDNESS (mg/ℓ)	ALKALINITY (mg/ℓ)	LANGLIER	TURBIDITY (NTU)	SUSPENDED SOLIDS (mg/ℓ)
Maximum	14,20	50,00	272,32	25,20	8,67	40.70	111,60	100	0,19	46,00	264
Minimum	4,80	22,00	13,84	4,80	7,00	28,70	58,70	56	-0,79	0,50	135
Average	8,45	33,09	130,45	18,81	7,89	31,55	80,43	75	-0,30	6,48	166
25 Percentile	7,40	29,00	76,71	16,00	7,64	28,70	63,70	65	-0,45	1,50	156
50 Percentile	8,10	32,00	132,60	18,65	7,96	29,65	78,40	68	-0,30	3,00	166
75 Percentile	9,20	39,00	167,53	24,00	8,07	34,35	94,70	86	-0,20	5,50	172

Table 4. Correlation coefficients (Spearman Rank) between bacterial counts and chemical/physical parameters.

	DOC	TOC	AOC	TOTAL CHLORINE	CONDUCTIVITY	HARDNESS	ALKALINITY	LANGLIER	TURBIDITY	SUSPENDED SOLIDS
Bacterial counts	0,800	0,1403	0,1073	-0,9487	0,9993	0,9890	0,4782	0.1082	0,4353	0,4290

Table 5. A summary of the biofilm development and composition of the bacterial population of the reticulation system of building no. 1.

WEEKS	MILD STEEL		GALVANIZED STEEL		COPPER		STAINLESS STEEL		PVC	
	Counts	Population	Counts	Population	Counts	Population	Counts	Population	Counts	Population
1	**Plate count:** $1,65x10^6$ **DAPI:** $5,80x10^7$	**Dominant:** *Pseudomonas* **Other:** *Acinetobacter*	**Plate count:** $3,23x10^5$ **DAPI:** $8,20x10^4$	**Dominant:** *Bacillus* **Other:** *Pseudomonas* *Micrococcus*	**Plate count:** $1,49x10^3$ **DAPI:** $4,20x10^4$	**Dominant:** *Bacillus* **Other:** *Pseudomonas*	**Plate count:** $9,44x10^4$ **DAPI:** $1,30x10^5$	**Dominant:** *Bacillus* **Other:** None	**Plate count:** $1,97x10^4$ **DAPI:** $3,10x10^4$	**Dominant:** *Achromobacter* **Other:** *Pseudomonas* *Bacillus*
2	**Plate count:** $1,81x10^7$ **DAPI:** $1,00x10^8$	**Dominant:** *Pseudomonas* **Other:** *Bacillus* *Staphylococcus*	**Plate count:** $6,14x10^4$ **DAPI:** ND	**Dominant:** *Bacillus* **Other:** *Pseudomonas* *Staphylococcus* *Micrococcus*	**Plate count:** $1,02x10^4$ **DAPI:** $9,20x10^4$	**Dominant:** *Staphylococcus* **Other:** *Aeromonas* *Pseudomonas* *Bacillus*	**Plate count:** $4,80x10^5$ **DAPI:** $5,90x10^4$	**Dominant:** *Bacillus* **Other:** None	**Plate count:** $4,01x10^4$ **DAPI:** $1,60x10^6$	**Dominant:** *Pseudomonas* **Other:** *Micrococcus* *Bacillus*
4	**Plate count:** $1,97x10^8$ **DAPI:** $1,40x10^8$	**Dominant:** *Deinococcus* **Other:** *Pseudomonas* *Bacillus* *Staphylococcus* *Micrococcus*	**Plate count:** $4,80x10^4$ **DAPI:** ND	**Dominant:** *Bacillus* **Other:** None	**Plate count:** $3,23x10^3$ **DAPI:** $8,40x10^4$	**Dominant:** *Bacillus* **Other:** *Pseudomonas* *Staphylococcus*	**Plate count:** $1,68x10^4$ **DAPI:** $5,08x10^6$	**Dominant:** *Bacillus* **Other:** None	**Plate count:** $2,05x10^4$ **DAPI:** $3,30x10^4$	**Dominant:** *Bacillus* **Other:** *Pseudomonas*
12	**Plate count:** $7,40x10^8$ **DAPI:** $3,00x10^7$	**Dominant:** *Staphylococcus* **Other:** *Pseudomonas* *Bacillus* *Micrococcus* *Deinococcus*	**Plate count:** $1,80x10^5$ **DAPI:** $1,10x10^6$	**Dominant:** *Pseudomonas* **Other:** *Bacillus*	**Plate count:** $1,49x10^4$ **DAPI:** $3,60x10^6$	**Dominant:** *Bacillus* **Other:** *Staphylococcus*	**Plate count:** $1,07x10^6$ **DAPI:** $5,80x10^5$	**Dominant:** *Bacillus* **Other:** None	**Plate count:** $7,47x10^5$ **DAPI:** $3,80x10^6$	**Dominant:** *Bacillus* **Other:** *Pseudomonas* *Acinetobacter*

ND - Not Done

Table 6. A summary of the biofilm development and composition of the bacterial population of the reticulation system of building no. 2.

WEEKS	MILD STEEL		GALVANIZED STEEL		COPPER		STAINLESS STEEL		PVC	
	Counts	Population	Counts	Population	Counts	Population	Counts	Population	Counts	Population
1	**Plate count:** $2,68x10^5$ **DAPI:** $5,70x10^7$	**Dominant:** *Pseudomonas* **Other:** *Bacillus*	**Plate count:** $3,07x10^4$ **DAPI:** $3,50x10^4$	**Dominant:** *Flavobacterium* **Other:** None	**Plate count:** $4,56x10^2$ **DAPI:** $7,10x10^4$	**Dominant:** *Pseudomonas* **Other:** None	**Plate count:** $5,11x10^5$ **DAPI:** $8,40x10^4$	**Dominant:** *Pseudomonas* **Other:** *Bacillus*	**Plate count:** $2,91x10^5$ **DAPI:** $7,50x10^4$	**Dominant:** *Pseudomonas* **Other:** None
2	**Plate count:** $4,40x10^5$ **DAPI:** $8,30x10^7$	**Dominant:** *Pseudomonas* **Other:** *Bacillus*	**Plate count:** $1,18x10^5$ **DAPI:** $1,60x10^5$	**Dominant:** *Pseudomonas* **Other:** *Flavobacterium* *Micrococcus*	**Plate count:** $2,59x10^4$ **DAPI:** $1,90x10^5$	**Dominant:** *Pseudomonas* **Other:** None	**Plate count:** $4,56x10^5$ **DAPI:** $5,00x10^4$	**Dominant:** *Bacillus* **Other:** *Alcaligenes* *Flavobacterium*	**Plate count:** $3,70x10^5$ **DAPI:** $1,30x10^5$	**Dominant:** *Pseudomonas* **Other:** *Staphylococcus* *Micrococcus*
4	**Plate count:** $2,05x10^6$ **DAPI:** $7,20x10^7$	**Dominant:** *Bacillus* **Other:** *Micrococcus*	**Plate count:** $5,51x10^4$ **DAPI:** ND	**Dominant:** *Flavobacterium* **Other:** Bacillus	**Plate count:** $3,93x10^3$ **DAPI:** $1,10x10^5$	**Dominant:** *Pseudomonas* **Other:** None	**Plate count:** $3,70x10^4$ **DAPI:** $3,56x10^6$	**Dominant:** *Bacillus* **Other:** *Serratia* *Pseudomonas*	**Plate count:** $4,09x10^5$ **DAPI:** $4,10x10^4$	**Dominant:** *Pseudomonas* **Other:** *Micrococcus*
12	**Plate count:** $4,72x10^7$ **DAPI:** $1,10x10^8$	**Dominant:** *Bacillus* **Other:** *Micrococcus*	**Plate count:** $1,02x10^5$ **DAPI:** $4,80x10^5$	**Dominant:** *Bacillus* **Other:** None	**Plate count:** $8,65x10^4$ **DAPI:** $4,50x10^6$	**Dominant:** *Micrococcus* **Other:** *Pseudomonas*	**Plate count:** $8,60x10^4$ **DAPI:** $4,20x10^5$	**Dominant:** *Bacillus* **Other:** None	**Plate count:** $6,35x10^4$ **DAPI:** $4,30x10^6$	**Dominant:** *Pseudomonas* **Other:** *Bacillus* *Micrococcus*

ND - Not Done

APPENDIX D

DIVISION OF WATER TECHNOLOGY, CSIR
HEALTH PROGRAMME

MICROBIAL GROWTH IN WATER DISTRIBUTION SYSTEMS: A LITERATURE REVIEW

BY
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PRETORIA
August 1989

1. INTRODUCTION

The growth of microorganisms in water distribution systems has been well documented throughout the world during the last century. Already in the 1930's the American Committee for Water Supply investigated the subject of bacterial growth in water distribution systems (American Committee on Water Supply, 1930). The Committee used a questionnaire which was sent to 90 cities in the U.S.A. and referred to growth of *Bacillus coli* (*Escherichia coli*). The research showed certain problems in the bacteriological quality of tap water in major cities. Since then, many other cases of microbial growth in water distribution systems and sudden increases in coliform counts in the final drinking water have been reported (Earnharht, 1980; Goshko *et al.*, 1983; Hudson *et al.*, 1983; Lowther & Moser, 1984). The phenomena of biofilm formation, or the attachment of microorganisms to the inner surfaces of the drinking water distribution system, has also been well documented (Allen, 1980; Touvinen *et al.*, 1980; Olson *et al.*, 1981; Touvinen & Hsu, 1982; Nagy & Olson, 1985).

In this literature study, attempts have been made to address different aspects of microbial growth in drinking water distribution systems. The study will review the following topics: the source of microbial growth in the system; the water distribution layout, with reference to structure and materials used; factors affecting microbial growth in the system, with special reference to total organic carbon levels and disinfection; health-related aspects of the microbial growth and the microbial quality of the final drinking water.

2. DRINKING WATER DISTRIBUTION SYSTEMS

Water distribution systems carry water from its source or treatment plant to the consumer. Systems differ much in their size; i.e. the area that they serve, the size of population and the distances the water is carried from its source. Differences in the size of the initial water works, the source of water and in the type of water works, may affect the final drinking water quality.

The ideal aim is to bring to the consumer potable water of an identical quality to that

leaving the treatment plant. However, it has been well documented that water leaving the waterworks is often of superior microbiological and sometimes chemical quality to that which reaches the consumer's tap.

2.1. Contamination of water in the drinking water distribution system

Different parameters may affect the quality of the drinking water during its passage through the distribution system. The drinking water distribution system is often not a totally closed or sealed entity. Due to this, a possibility of external contamination exists. Hazardous situations, in which the system may be polluted, may arise accidentally or more rarely, intentionally. The water distribution system should be designed to prevent external contamination. Service reservoirs, which are used as storage units for potable water, may constitute a weak link in the layout of water supply distribution systems. Often reservoirs are not completely sealed, and in some cases are open. If the tanks or reservoirs are underground, possibly in close proximity to sewage, cross contamination due to sewage overflow may occur. Open reservoirs may be contaminated by birds (especially gulls), flying insects, and algal growth due to day light (Jones *et al.*, 1978).

In some apartment buildings, roof reservoirs have been built. If such reservoirs are not completely covered, contamination by rain can occur. Heavy bacterial contamination, including *E. coli* and other coliforms of different origins (bird as well as domestic pets), has been shown. This is especially true after the first heavy rainfall following a dry spell (Anon, 1984).

It is recommended that reservoirs for storage of potable water be situated and built in such a way as to lower the risk of sewage or any other contaminants being introduced to the water distribution system.

2.2. Contamination of drinking water via back siphonage and cross connections

Back siphonage can be defined as the flow against the normal flow direction of the water supply network. Reduction of pressure of waterflow in the system and other parameters involved in the layout of the plumbing system may result in back siphonage. Substantial microbial contamination may result, depending on the system outlay. A study carried out by Gilfillan (1971) in England, showed that although 85% of the properties studied posed a risk of back siphonage as assessed in terms of the specific requirement, very low probability of actual occurrence of back siphonage was reported.

Cross connection may be defined as the connection between pipes carrying domestic water supply and other pipes carrying water or any other liquid of another source. Water distribution systems in industrial areas in particular are at risk, but plumbing of private households is sometimes also affected. Accidental connection between potable and discharged waters is rare.

2.3. Material usage and its effects on the microbiological quality of potable water supply

It has been known for many years that material used for the construction of the potable water distribution systems can adversely affect the quality of water. Until the 1950's most water distribution systems were built largely of materials such as wooden pipes, cast iron or steel pipes with a protective lining made of either bitumen or coal tar. Leaching of heavy metals to the water distribution system is also well known (De Mora *et al.*, 1987). Joint and seal material was almost exclusively made from natural substances such as leather, juta, cork, string or natural rubber. Lubrication was achieved using linseed oil, soaps or tallow. During this period many incidents of microbial pollution of potable water could be attributed to the utilization of these natural materials (Taylor, 1947; Burman & Colbourne, 1976; Schoenen & Schöler, 1985).

From the 1950's onwards, mainly synthetic materials have been used in the construction of potable water distribution systems. Most are derived from the oil industry. According to Colbourne (1985), the change in the type of material used has disregarded to a great extent the support provided by such materials for bacterial growth or its effects on the microbial and chemical quality of the final water. By the mid 1960's and early 1970's studies had shown growth of bacteria on glass reinforced tanks and on polyvinylchlorine (PVC), as well as on an epoxy used for lining (Burman & Colbourne, 1976; Schoenen & Schöler, 1985).

In England a microbial growth test and a toxicity test was implemented in order to test the suitability of materials for use in the water industry, including water distribution (Ashworth & Colbourne, 1987).

2.4. Dead ends and their contribution to microbial growth

The layout of water supply distribution systems is often such that dead ends, in which very little flow occurs, are unavoidable. Accumulation of debris occurs in such dead ends where no free chlorine is available. Bacteria and fungi can utilize the debris for growth and development, which can eventually lead to the deterioration of the water quality.

The problem can be overcome by designing systems to avoid dead ends. Where dead ends already exist, proper flushing should take place and regular sampling should be carried out (Anon, 1984).

3. MICROBIAL GROWTH IN THE WATER DISTRIBUTION SYSTEM

3.1. Definitions of microbial growth in the system

Regrowth - recovery of disinfected injured bacteria originating in the water treatment plant.

Injured organisms - a form of reversible injury. Often coliforms in an injured state do not form colonies on m-Endo agar Les, but may be recovered on a special medium developed for injured coliforms.

Aftergrowth - microbial growth in the water distribution system. There are two mechanisms which form the basis for bacterial presence in water distribution systems: (a) breakthrough-bacteria escaping disinfection (or injured bacteria); (b) growth. The two are often related since without initial breakthrough no growth will occur in the system. Different factors will support breakthrough and growth in the system. Excessive microbial growth will result in: (a) unhygienic water which is a health hazard; (b) deterioration of the pipe material, leading to increased growth; (c) tubercles which will cause flow friction, flow resistance and increase pumping costs; (d) taste and odour problems.

Excessive microbial growth often appears in the system as a formation of biofilms.

Biofilms - microbial cells which are attached to pipe surfaces, to extra polymeric substances, tubercles (mineral deposits) or to sediment deposits. McFeters (1984) defined biofilms as microorganisms and their extracellular products associated with a substratum.

Planktonic cells - bacterial cells which are unattached and may flow with the stream of water, and appear in the final drinking water.

3.2. The influence of the nature of the raw water and water treatment processes on microbial growth in the water supply distribution system.

In order for bacteria to grow in the water distribution system, they must be introduced to the system. Therefore, the raw water must contain bacteria which must escape the water treatment process if growth is to occur.

The level of minerals or other nutrients in the water is of great importance to microbial growth. Raw waters differ in their mineral composition and levels of organic carbon.

Although most raw water used as a source for public water supplies will contain adequate amounts of minerals which will support bacterial growth, the specific mineral composition will determine which type or species of bacteria occur (Anon, 1984). Raw waters also differ greatly in their levels of organic carbon. Ground water is often very low in organic matter while surface waters generally are rich in organic matter and will support substantial microbial growth. The major part of the organic matter in the raw waters consists of humic substances which are usually resistant to biodegradation and are utilized very slowly for bacterial growth. Usually, only specialized bacteria are capable of biodegrading humic substances while many other bacteria are capable of utilizing the biodegradation products.

Different water treatment processes will contribute to the removal of bacteria and organic matter from the raw water.

Alum coagulation - will remove humic substances by inclusion in the alum floc.

Slow sand filtration - provides suitable conditions for growth of bacteria which will biodegrade organic matter. In some water works, a chlorinated backwash step is included in the water treatment to reduce bacterial growth. This step often results in increased growth of bacteria in the water distribution system as more organic matter enters the system.

pH and lime treatment - The results of a study by Martin and co-workers (1982) indicate a delicate balance between bacterial growth in the water distribution system, and the pH of the water and the level of the chlorine residual. The effect of the pH might be dominant. Increase of pH by lime addition was shown to be effective in the elimination of coliform growth in the water distribution system.

Disinfection - the final step in most water treatment processes is an oxidation process, by either chlorination or ozonation. This step is introduced to remove bacteria or other microorganisms which escape prior treatment processes. As the disinfection is not a sterilization process, viable or injured microorganisms may escape this step and multiply in the water distribution system. The oxidation process, while removing or partially removing microorganisms, also oxidizes any remaining organic matter not

biodegraded in the previous treatment steps. This yields a more readily available substrate, which can be easily utilized by microorganisms as an energy source. Thus ozonation or chlorination may increase microbial growth in the system. In order to avoid such growth the disinfection step should produce only enough residual disinfection to prevent growth in the system (Anon, 1984).

3.3. Relationship between turbidity and microbial quality of water in the system

Turbidity has been used as a measure of water quality for many years. In the U.S.A. a limit of 1 NTU has been set for water leaving the water treatment plant (EPA, 1976), but no regulations for the water leaving or within the water distribution system are available.

Herson and co-workers (1984) showed that turbidity correlates with the presence of nutrients in the system. The presence of nutrients may result in microbial growth and deterioration of water quality. Ridgeway and Olson (1982) showed that increased turbidity relates to high chlorine demand and decreased availability of residual disinfectant in the water distribution system. LeChevallier and co-workers (1984) suggested a relationship between high levels of turbidity and increased growth. Increased turbidity means an availability of a matrix for the transport of microorganisms through the system or a way of introducing the microorganisms.

However, recent studies showed that the usefulness of turbidity as an indicator of bacteriological quality is at best variable (Reilly & Kippin, 1983), and most distribution systems show only a relatively weak correlation between microbial quality and turbidity (McCoy & Olson, 1986).

Growing evidence suggests that turbidity as a standard may be inadequate as a predictive measure of the microbial safety of a water supply. McCoy and Olson (1986) studied the relative role of turbidity and particle sizes, counts and distribution, as well as bacterial counts, in chlorinated and unchlorinated water distribution systems. The change in these parameters, and their relationship as water moves from the source to the consumer, were investigated. They concluded that although turbidity and particle counts showed a direct positive relationship, this is not consisting throughout the

system due to variability in the measurements. Their most important conclusion was that there is no predictive relationship between bacteriological quality and turbidity or particle size in the system, with the exception of events where high numbers of bacteria (breakthrough) were shown.

3.4. **Biofilm formation in the water supply water distribution system**

The phenomenon of attached microorganisms and microbial growth on the inner surface of drinking water distribution systems is well documented (Allen, 1980; Touvinen *et al.*, 1980; Ridgeway & Olson, 1981; Touvinen & Hsu, 1982; Nagy & Olson, 1985; LeChevallier *et al.*, 1987). Attached microbial populations have been sampled and measured in a variety of ways. Studies include mechanisms of attachment, type of bacteria attached to different surfaces, density of bacteria attached, factors affecting attachment and detachment, and characteristic qualities of bacteria grown in biofilm, eg. increased chlorine resistance. Bryers and Characklis (1982) indicate a strong relationship between the biofilm formation rate and factors such as transport and attachment of cells to the pipes; cell reproduction rate; bioproduct formation and availability; detachment.

The main factor which affects the extent of biofilm growth is the substance loading rate in the system. Characklis (1988) has formulated an equation in which different parameters are related to the substrate loading rate (Ns). Ns is dependent upon the concentration of the substrates (S), the volumetric flow rate in the pipe (F), the surface area of the pipe (A), the mean fluid velocity (V), the pipe length (L) and the pipe diameter (d).

$$Ns = \frac{SF}{A} = \frac{Svd}{4L}$$

According to this equation, 900 g of biofilm per day can be produced in a pipe of 4,65

m diameter where the flow rate is 500 ℓ/min and the total organic carbon (TOC) is 0,6 mg/ℓ. This mass is equivalent to 10^5-10^7 cells/100 mℓ of water.

3.4.1. Total organic carbon (TOC) and assimilable organic carbon (AOC)

The carbon which is easily available to the microorganisms to utilize as an energy source is calculated as AOC. The value of AOC is often 4-20% of the TOC in the system. One milligram of AOC per litre was calculated as equal to 10^5-10^7 bacterial cells per 100 mℓ. Thus low levels of assimilable organic carbon can support high populations of microbial growth in the water distribution system. Van der Kooij and co-workers (1982a) showed a correlation between levels of AOC and microbial growth in the water, also defined as the aftergrowth potential of the water. An equivalent of growth potential may be calculated by growing a strain of *Pseudomonas* bacteria in sterilized water samples and measuring the formation of acetate carbon (Van der Kooij *et al.*, 1982a). According to Van der Kooij and co-workers (1982b), bacterial aftergrowth is limited to AOC levels of less than 50 µg of acetate carbon equivalent per litre of water. Kemmy and co-workers (1988) introduced a modification to the Van der Kooij method and built a system in which mixtures of 2-4 different bacterial strains are used for inoculating the sterile water samples. Van der Kooij and Hijnen (1988) also demonstrated the capability of *Klebsiella pneumoniae* to grow in water containing low concentrations of substrates.

3.4.2. Bacterial attachment to pipe surfaces

The physical/chemical forces by which bacteria may attach to surfaces have been studied. Attachment of bacterial cells to a solid surface may provide a more favourable environment for bacterial cell growth. A living organism, such as a bacterial cell, can produce material to change its surface properties, and the properties of material surfaces nearby (Lion *et al.*, 1988). Some bacteria may adhere by hydrophobic interaction while others form well-defined appendages to assist their attachment. These may include holdfast and pili. The majority of the bacteria will attach to surfaces or each other by exopolymers. According to Geesey and co-workers (1988) bacteria bind

metal ions by extracellular polymers. Bacteria form chemically and structurally unique acidic polysaccharides (which form the exopolymers) with affinities to different metal ions (Mittelman & Geesey, 1985).

According to Tamper (1987), there are several mechanisms or factors which enable bacteria to attach to surfaces, including Van der Waal's forces, ionic binding, covalent coupling, cross-linking in gel, microcapsules and fibre.

Lion and co-workers (1988) refer to two phases of bacterial attachment, the first being instantaneous and reversible while the second is time-dependent and irreversible. The first step is explained as repulsion-attraction mechanisms involving Van der Waal's forces and electrical double layer forces. Formation of the extracellular polymeric fibrils occurs during the second step.

Attachment of bacterial cells and biofilm formation in the water distribution system was shown by Ridgeway and Olson (1981). Using scanning electron microscopy, these investigators studied the nature and extent of the association of microorganisms with pipe surfaces. They documented attachment mediated by extracellular fibrillar appendages and showed particles of 10-50 μm to be colonized with $10\text{-}10^6$ bacterial cells per particle.

It has also been shown that pipe materials will support attachment to different degrees (Colbourne, 1985). *Legionella* bacteria were shown to prefer rubber and to adhere to silicon and stainless steel, while attachment to copper was minimal (Schofield & Locci, 1985). Haudidier and co-workers (1988) showed bacterial biofilm formation on cement and PVC materials while other studies indicated that under laboratory conditions bacterial growth in the form of a biofilm may also occur on glass surfaces (LeChevallier *et al.*, 1988).

4. TYPES OF MICROORGANISMS GROWING IN THE DRINKING WATER DISTRIBUTION SYSTEM

Microorganisms, especially bacteria, growing in water distribution systems are often

difficult to characterize. These microorganisms are difficult to cultivate in pure cultures and the use of biochemical identification patterns often results in a classification of non-precise taxonomical groupings (Anon, 1984).

Groups of microorganisms which have been identified thus far include *Flavobacterium*, *Pseudomonas*, *Aeromonas*, *Arthrobacter*, *Caulobacter*, coliforms, *Klebsiella*, *Bacillus*, *E. coli*, *Enterobacter*, *Citrobacter*, *Acinetobacter*, *Prosthescomicrobium*, *Alcaligenes*, *Serratia* and *Actinolegionella* (Van der Kooij & Zoetemann, 1978; Ridgeway & Olson, 1981; Martin *et al.*, 1982; Olson, 1982; Olivieri *et al.*, 1985; Schofield & Locci, 1985; Herson *et al.*, 1987). *Cytophaga*, actinomycetes, yeasts, many iron bacteria and sulphate reducers were found to colonize in the system (Touvinen *et al.*, 1980; Ridgeway & Olson, 1981).

By the use of scanning electron microscopy Ridgeway and Olson (1981) observed groups of autotrophic iron-oxidizing bacteria, *Gallionella*, in biofilm formation. The bacterium was recognized by its characteristic spiral stalk, composed of several delicately wound protein fibrils. *Gallionella* have also often been isolated from potable water. The bacteria obtain their energy chemolitotrophically, presumably by enzymatic oxidation of soluble ferrous iron to the ferric state (Kuznsetsow, 1970). Other studies showed tubercles containing other chemolitotrophic bacteria, such as sulphate reducers, nitrate reducers and nitrate, ammonia and sulphate oxidizing bacteria (Touvinen *et al.*, 1980; Touvinen & Hsu, 1982).

Ridgeway and Olson (1981) also observed other filamentous bacteria, such as the genus *Prosthecomicrobium*, with cell sizes of up to 1 µm in diameter. Members of this genus are often found in low nutrient aquatic environments (Stanley, 1968).

Martin and co-workers (1982) have shown isolation of coliform bacteria from the water distribution system of Nova Scotia in the summer of 1977. The majority of the coliforms were identified as *Klebsiella*. *Klebsiella pneumoniae* was also isolated by other researchers from water distribution system biofilms as well as from drinking-water (Seidler *et al.*, 1977; Clark *et al.*, 1982; Olivieri *et al.*, 1985; Geldreich & Rice, 1987).

Edge and Finch (1987) isolated *Aeromonas hydrophila* in large numbers from potable

water in East Anglia. LeChevallier (1982) isolated *Aeromonas sobria* from drinking water.

In recent years fungi have been isolated from water distribution systems in different parts of the world, including the U.K., North America, Finland and France (Bays *et al.*, 1970; Nagy & Olson, 1982; Niemi *et al.* 1982; Hizelin & Block, 1985). The fungi isolates are often soil hypomycetes, which are imperfect fungi. Although at first the fungi presence in the water was assumed to be the result of contamination by airborne spores, research by Rosenzweig and Pipe (1988) indicated that the numbers of fungi detected in potable water of the water distribution system were too high to be explained by adventitious spores in the system. Initial levels of residual chlorine reported were sufficient to eliminate fungal spores, but the high demand for chlorine in the system and the resultant low level of residual disinfectant provided conditions conducive to the development of fungal spores.

Another microorganism which was recently found to colonize the water distribution system, especially the hot water system, is the bacterium *Legionella pneumophila.* These bacteria were also shown to be present in potable and domestic hot water. (Dennis *et al.*, 1982; Best *et al.*, 1983; Schofield & Locci, 1985; Colbourne *et al.*, 1988). Warm temperature, stagnation and the use of unsuitable fixtures and fittings can provide favourable conditions 'suitable' for the growth of Legionella bacteria in the water distribution system (Barrow, 1986).

5. SURVIVAL OF MICROORGANISMS IN THE WATER DISTRIBUTION SYSTEM

5.1. Detection of bacteria in the water distribution system

The total count of bacteria in drinking water is often used for the evaluation of microbiological quality of drinking water. Cliver and Newman (1987) found methodologies differing from country to country. It was found that although nutrient agar was used in West Germany, peptone agar was the most commonly used in France, yeast extract agar in the U.K. and tryptone agar in Canada. Growth conditions

such as incubation temperatures and the period of incubation also differ. In some countries the incubation period used is 24 h or 48 h at 37 °C, or 72 h at 20-22 °C. Only in certain countries is a combination of both 37 °C and 22 °C used. As a result of the different enumeration procedures the final results also differ. New isolation methods, in which growth agar and conditions vary, were developed by Fiksdal and co-workers (1982) and Reasoner and Geldreich (1985).

The most direct method for detection of viable bacteria in water is the use of the epifluorescence technique using acridine orange. McCoy and Olson (1986) compared the method for the detection of heterotrophic bacterial plate counts using Reasoner's R2A low nutrient agar medium (Reasoner & Geldreich, 1985) and incubation of plates at 23 °C for 7 days with the epifluorescent direct cell counting technique (Daley, 1979). The results of this study indicate the heterotrophic plate count to underestimate the epifluorescent direct cell counts by a factor of at least 500, but showed a direct proportionality between the two methods. Some studies report coliforms in the system and relate the absence of coliforms in the water tested directly after treatment to injured bacteria which may be recovered on mT7 agar rather than the commonly used m-Endo (LeChevallier *et al.*, 1983; LeChevallier *et al.*, 1987). Hence, using different methodology different numbers of microorganisms can be isolated from the same drinking water sample.

5.2. Survival mechanisms of bacteria in the water distribution's chlorinated water

Experiments have shown that bacteria when attached to surfaces show greater resistance to disinfection (LeChevallier *et al.*, 1988). A 150-fold increase in resistance to disinfection over unattached bacteria was found with *Klebsiella pneumoniae* when growing on glass microscope slides in a high nutrient agar. Hersen and co-workers. (1987) studied the effect of attachment of *Enterobacter cloacae* to particles in water distribution systems on its resistance to chlorination. They concluded that the attached organisms were highly resistant to chlorine. Attachment of organisms to surfaces has been shown to alter their physiology. Attached organisms were found to be generally more active in absorbing nutrients as well as more resistant to environmental stress

such as starvation, heavy metals and chlorine (Stotzky, 1967; Hudson *et al.*, 1983; Backer, 1984; LeChevallier 1984). Olivieri and co-workers (1985) reported coliform recovery in free residual chlorine concentrations of 5-8 mg/ℓ in the water distribution system, whereas the same bacterial culture showed no difference in resistance pattern to other bacteria when grown unattached to any surface. The question of the mode of action in which the chlorine resistance of a biofilm is achieved, is still unanswered. Whether the chlorine is consumed (high chlorine demand in the system was shown) or simply does not penetrate the biofilm still remains to be determined.

Other factors that were shown to influence disinfection resistance are the age of the biofilm, bacterial encapsulation and previous growth conditions (growth medium and growth temperature).

The type of residual disinfectant in use showed an influence on the type of resistance mechanism observed. The efficacy of disinfection by free chlorine was affected by bacterial attachment to surfaces, age of the biofilm encapsulation and nutrient levels. Wolfe and co-workers (1985) found that a number of bacterial genera found in chlorinated water demonstrated a variety of disinfection resistant patterns to free chlorine and monochloramines.

Ward and co-workers (1982) reported that *Flavobacterium* strains were more sensitive to monochloramine than free chlorine. The efficacy of disinfection by monochloramines depends on the attachment of the bacterial cells to surfaces.

5.3. Additional factors limiting growth of bacteria in the water distribution system

Martin and co-workers (1982) showed growth of bacteria in water to be highly influenced by the pH of the water. Fifty percent of the organisms tested survived only 1 hour at pH 9.

Rosenzweig (1987) showed the importance of two phosphate based corrosion controlling chemicals in the distribution system. He studied the influence of phosphate

addition on the microbial growth in the system and found no stimulated coliform growth.

In another study the role of bicarbonate in bacterial growth in drinking water was investigated (Fransolet *et al.*, 1988). The results of this study indicated that several strains of heterotrophic bacteria can assimilate inorganic carbon significantly, and may develop in bicarbonated water even when the organic content is low. Potassium was also observed as a limiting factor in the microbial growth.

6. HEALTH RELATED ASPECTS OF MICROBIAL GROWTH IN THE WATER DISTRIBUTION SYSTEM

Large numbers of heterotrophic bacteria can be isolated from drinking water systems by using standard microbiological techniques. Many of these bacteria have been shown to be human secondary opportunistic pathogens. In recent years growth of fungi and bacteria in the water distribution system has been shown to be of significance to human health. Different studies have shown the presence of opportunistic pathogens and fungi in water distribution systems throughout the world. Some of these are discussed below.

Apart from direct health hazards, the growth of bacteria and fungi in the system can introduce another problem, as their presence may mask other indicator organisms resulting from a real breakthrough of the treatment process.

6.1. *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is an opportunistic bacteria which may cause eye and ear infections, as well as infection of wounds and burns. Studies have shown contamination of ice cubes with the bacteria to be the causative agent for serious post-operative infection in tonsillectomy patients and in hospital burn units (Louwbury *et al.*, 1970; Newson, 1968). The bacterium was also found to produce an enterotoxin which results in severe diarrhoea (Anon, 1984). So far no limits for *P. aeruginosa* in water are

available, but the German drinking water legislation considers the bacterium to be a human pathogen.

6.2. **Aeromonas hydrophila**

Aeromonas hydrophila was found in water distribution systems in East Anglia (Edge & Finch, 1987). It is well-documented that these organisms are capable of causing disease in man (Hauson, 1977). *Aeromonas* spp. also pose an increased risk for patients undergoing renal dialysis. Some strains of *Aeromonas* produce enterotoxins which cause severe gastroenteritis (Rosner, 1964). The rate of production and stability of the toxin in water is as yet unknown (Edge & Finch, 1987). Moyer (1987) reported *Aeromonas* spp. to cause acute diarrhoea in children and traveller's diarrhoea in adults.

6.3. ***Klebsiella pneumoniae***

Klebsiella pneumoniae has been reported to be a dominant coliform organism in water distribution systems (Seidler *et al.*, 1977; Clark *et al.*, 1982; Geldreich & Rice, 1987). The bacteria grows in sediments which provide a level of protection from disinfection (Martin *et al.*, 1982). *Klebsiella pneumoniae* is a pathogen which can indicate the presence of faecal pollution and has been shown to cause inflammation of the lungs.

6.4. ***Enterobacter cloacae***

Enterobacter cloacae is the most frequently isolated *Enterobacter* species from man and animal. It is an opportunistic pathogen isolated from urine, sputum, the respiratory tract, puss and occasionally from blood and spinal fluid. It has been shown to be of increasing importance in hospitals, especially in intensive care units, emergency units and urology units (Krieg & Holt, 1984). Victoreen (1977) isolated *E. cloacae* from tubercles of water distribution systems.

6.5. *Legionella pneumophila*

Legionella pneumophila is also a known human opportunistic bacterial pathogen which can cause either Legionnaire's disease (a pneumonia-like disease) or pontiac fever. Studies have shown *Legionella* bacteria to be present in potable water and a correlation has been shown between bacterial presence in domestic hot shower systems and pneumonia cases in populations studied (Dennis *et al.*, 1982).

6.6. *Flavobacterium*

Flavobacterium spp. were isolated from water distribution biofilms (LeChevallier *et al.*, 1987). Species of *Flavobacterium* are recognized as the cause of neonatal meningitis, meningitis in adult, bacteremia and respiratory tract infections. *Flavobacterium* are known as opportunistic pathogens which will infect the seriously ill (Krieg & Holt, 1984).

6.7. *Other bacteria*

Bacteria such as *Moraxella*, *Acinetobacter*, *Bacillus*, *Alcaligenes* and *Achromobacter* have been recovered from drinking water distribution systems. All these genera include species which are known opportunistic human pathogens.

Another bacterium isolated by Victoreen (1977) is *Serratia marcescens* which is a prominent opportunistic pathogen in hospitalized burn patients. Other *Serratia* spp. can cause bacteremia (Krieg & Holt, 1984).

Olson (1982) isolated *E. coli* bacteria from biofilm material in mortar lined pipes of the water distribution system. These bacteria are normally used to indicate the possible contamination of water by faecal matter. The species are also known to contain enterotoxigenic strains (Camper *et al.*, 1985).

6.8. Fungi

Growth of fungi in the water distribution system has been shown to be a health hazard as a result of the formation of mycotoxins and of allergies. Acute toxins, such as mycotoxins have been associated with the growth of fungi on food. Exposure to fungal spores while using hot baths has been shown to cause respiratory symptoms (Atterholm *et al.*, 1977). Antibodies against fungus grown were shown in people using baths (Anon, 1984).

6.9. Pathogens

Camper and co-workers (1985) showed attachment of pathogenic bacteria such as *Yersinia enterolitica*, *Salmonella typhimurium* and enterotoxigenic *E. coli* to particles of a granular activated carbon bed. They also showed that pathogens may attach to particles in a mature biofilm, thus indicating a possibility of survival and presence of such pathogenic bacteria in the water distribution system.

References

Allen, M.J. (1980). The occurrence of microorganisms in water main encrustations. *Journal of American Waterworks Association*, **72**, 614.

American Committee on Water Supply, (1930) Bacterial aftergrowth in water distribution systems. *American Journal of Public Health*, **20**, 485-491.

Anonymous, (1984) Drinking Water Microbiology, Committee on the challenges of modern society (Nato/CCMS, CCMS 128, Pilot Project Services 1984).

Ashworth, J. and J.S. Colbourne (1987). Microbial alteration of drinking water by building services materials - field observation and the U.K. water fitting testing scheme. In *Proceedings of the Biodetermination of Construction Materials*, Biodetermination Society 1987.

Atterholm, I., Hallberg, T. Ganrot-Norlim, K. and Ringertz, O. (1977) Unexplained acute fever after a hot bath. *Lancet ii*, 684-686.

Backer, K.H. (1984) Protective effect of turbidity on *E. coli* during chlorine disinfection.

In *Water quality and the Public Health.* eds. Crusberg, T.C., Gheetham, R.D. and Hoffman, A.H., Worcester Consortium for Higher Education, Worcester, Massechutes.

Barrow, G.I. (1986). Legionnaire's disease and its impact on water supply management. Presentation in Scientific Section of the IWES, February 1986, Huntington.

Bays, L.R., N.P. Burman and W.M. Lewis (1970). Taste and odours in water supplies in Great Britain: A survey of the present position and problems for the future. *Water Treatment and Examination*, **19**, 136-160.

Best, M., J. Stout, R.R. Murder, V. Yu, A. Goetz, F. Taylor (1983) Epidemiological link with disease and evaluation of a method for control of nosocomial Legionnaire's disease and Pittsburgh pneumonia *Lancet ii*, 307-310.

Bryers, J.P. and W.G. Characklis (1982) Process governing primary biofilm formation. *Biotechnology and Bioengineering*, **24**, 2451-2476.

Burman, N. and Colbourne, J. (1976) The effect of plumbing material on water quality. *Journal of Institute of Plumbing*, **3**, 12-13.

Camper, A.K., LeChevallier, M.W., Broadway, S.C. and McFeters, G.A. (1985) Growth and persistence of pathogens on granular activated carbon filters. *Applied and Environmental Microbiology*, **50**, 1378-1382.

Characklis, W.G. (1988) Bacterial population in potable water distribution systems. In *Bacterial Regrowth in Distribution Systems.* Research Project for AWWA Research Foundation.

Clark, J.A., Burger, C.A. and Sabatinos, L.E. (1982) Characterization of indicator bacteria in municipal raw water, drinking water and new main water. *Canadian Journal of Microbiology*, **28**, 1002-1013.

Cliver, D.O. and Newman, R.A. (1987) Committee on the Challenges of Modern Society (NATO/CCMS) 123. Special issue, Drinking Water Microbiology, *Journal of Environmental Pathology, Toxicology and Oncology*, **7**, 82.

Colbourne, J.S. (1985) Material usage and their effects in the microbiological quality of water supplies. *Journal of Applied Bacteriology*, Symposium Supplement, 475-595.

Colbourne, J.S., Dennis, P.J., Trew, R.M., Berry, C. and Vesly, G. (1988) Legionella and Public water supplies. *Water, Science and Technology*, **20**, 5-10.

Daley, R.J. (1979) Direct epifluorescence enumeration of native aquatic bacteria : uses,

limitations and comparative accuracy. In *Native Aquatic Bacteria: Enumeration, Activity and Ecology.* ed. Costerton, J.W. and Colwell, P.R. pp. 29-45, American Society for Testing and Materials. ASTM STP 695.

De Mora, S.J., Harrison, R.M. and Wilson, S.J. (1987) The effect of water treatment on the speciation and concentration of lead in domestic tap water derived from a soft upland source. *Water Research*, **21**, 83-94.

Dennis, P.J., Taylor, J.A., Fitzgeorge, R.B., Bartlett, C.R.L. and Barrow, G.I. (1982) Legionella pneumophila in water plumbing systems. *Lancet i*, 949-951.

Earnharht, K.B. Jr (1980) Chlorine resistance coliform: The Muncie Indiana experience paper 4-2, AWWA, Water Quality Technical Conference, Miami Beach, Florida.

Edge, C.J. and Finch, P.E. (1987) Observation on bacterial after growth in water supply distribution systems: Implication of disinfection strategies. *Journal of the Institute of Water and Environmental Management*, **1**, 104-110.

EPA, (1976) National Interim Primary Drinking Water regulation, EPA-570/9-76003. Office of Water Supply, U.S. EPA, Washington D.C.

Fiksdal, L., Vik, E.A., Mills, A. and Staley, J.T. (1982) Non-standard methods for enumerating bacteria in drinking-water. *Journal of American Water Works Association*, **74**, 313-318.

Fransolet, G., Depelchin, A., Villiers, G.,Goossens, R. and Marsschellin, W.J. (1988) The role of bicarbonate in bacterial growths in oligotrophic water. *Journal of the American Water Works Association*, , 57-61.

Geesey, G.G., Jong, L., Jolley, J.G., Hankins, M.R., Iwaoha, T. and Griffiths, P.R. (1988) Binding of metal ions by extracellular polymers of biofilm bacteria. *Water Science and Technology*, **20**, 161-165.

Geldreich, E.E. and Rice, E.W. (1987) Occurrence, significance and detection of *Klebsiella* in water systems. *Journal of the American Water Works Association*, **79**, 74-80.

Gilfillan, P.J. (1971) *Report on the investigation of backsiphonage risks in domestic properties*, Winter 1970/71, Report TP82, Water Research Association Medmenham, England.

Goshko, M.A., Pipes, W.O. and Chrisitina, R.R. (1983) Coliform occurrence and chlorine residual in small water distribution systems. *Journal of the American Water Works Association*, **74**, 371-374.

Haudidier, K., Paquin, J.L., Francais, T., Hartemann, P., Graphin, G., Colin, F.,

Jourdain, M.J., Block, J.C., Cheron, J., Pascal, O., Levi, Y. and Miazga, J. (1988) Biofilm growth in drinking water network: A preliminary industrial pilot plant experiment. IAWRC, Irvine California, February 8-11.

Hauson, P.G. (1977) Freshwater wound infection due to *Aeromonas hydrophila*. *American Medical Association*, **233**, 1053-1054.

Herson, D.S., Marshall, D.S. and Victoreen, H.T. (1984) Bacterial persistence in the water distribution systems. *Proceedings of the American Water Works Association Technical Conference*, **12**, 309-322.

Hersen, D.S., McGonighe, B., Payer, M.A. and Baker, K.H. (1987) Attachment as a factor in the protection of *Enterobacter cloacae* from chlorination. *Applied and Environmental Microbiology*, **53**, 1178-1180.

Hizelin, F. and Block, J.C. (1985) Yeast and filamentous fungi in drinking-water. *Environmental Technology Letters*, **6**, 101-106.

Hudson, L.O., Harklin, J.W. and Battaglia, M. (1983) Coliforms in water distribution system - a remedial approach. *Journal of the American Water Works Association*, **75**, 564-568.

Jones, F., Smith, P. and Watson, D.C. (1978) Pollution of a water supply catchment by breeding gulls and the potential environmental health implications. *Journal of the Institute of Water Engineers Science*, **32**, 469-482.

Kemmy, F.A., Fry, J.C. and Beach, R.A. (1988) Development and operational implementation of a modified and simplified method for determination of DOC in drinking water. IAWPRC, Brighton, July 1988.

Krieg, N. and Holt, J.G. (1984) *Bergey's Manual of Systematic Bacteriology*, vol. 1. London: Williams & Wilkins.

Kuznsetsow, S.I. (1970) The iron and manganese cycle in lakes. In *The microflora of lakes and its geochemical activity*. ed. Oppenheimer, C.H., pp. 365-407. Austin: University of Texas Press.

LeChevallier, M.W. (1982) *Aeromonas sobria* in chlorinated distribution water supplies. *Microbial Ecology*, **8**, 325-333.

LeChevallier, M.W., Cameron, J.C. and McFeters, G.A. (1983) New medium for improved recovery of coliform bacteria from drinking water. *Applied and Environmental Microbiology*, **45**, 484-492.

LeChevallier, M.W., Hassenauer, T.S., Camper, A. and McFeters, G.H. (1984) Disinfection of bacteria attached to granular activated carbon. *Applied and*

Environmental Microbiology, **48**, 918-923.

LeChevallier, M.W., Babcock, T.M. and Lee, R.G. (1987) Examination and characterization of distribution system biofilms. *Applied and Environmental Microbiology*, **53**, 2714-2724.

LeChevallier, M.W., Cawthon, C.D. and Lee, R.G. (1988) Mechanism of bacterial survival in chlorinated water. International Specialized Conference, IAWRC, Microbiology of Waters and Wastewaters, Irvine California, February 8-11, 1988.

Lichtigfeld, S. and Melmed, L.N. (1982) The suitability of m-Endo-LES agar for total coliform counts by membrane filtration. *Water S.A.*, **8**, 173-177.

Lion, L.W., Shuler, M.L., Hsieh, K.M. and Ghiorse, W.C. (1988) Trace metal interaction with microbial biofilm in natural and engineered systems. *Environmental Control*, **17**, 273-306.

Louwbury, E.J.L., Thom, B.T., Lilly, H.A., Rabb, J. and Whittal, A. (1970) Sources of infection with *Pseudomonas aeruginosa* in patients with tracheostomy. *Journal of Medical Microbiology*, **3**, 39.

Lowther, E.D. and Moser, R.H. (1984) Detecting and eliminating coliform regrowth. AWWA, Water Quality Technical Conference, Denver, paper 413-4.

Martin, R.S., Gates, W.H., Tobin, R.S., Granthan, D., Junarah, R., Wolfe, D. and Forestall, P. (1982) Factors affecting coliform bacteria growth in distribution systems. *Journal of the American Water Works Association*, **74**, 34-37.

McCoy, W.F. and Olson, B.H. (1986) Relationship among turbidity, particle counts and bacteriological quality within water distribution lines. *Water Research*, **20**, 1023-1029.

McFeters, G.A. (1984) Biofilm development and its consequence. In *Microbial adhesion and Aggregation*, ed. Marshall, K.C., pp. 109-124. Berlin: Springer Verlag.

Mittelman, M.W. and Geesey, G.G. (1985) Copper binding characteristics of exopolymers from a freshwater sediment bacterium. *Applied and Environmental Microbiology*, **49**, 846-851.

Moyer, N.P. (1987) Clinical significance of *Aeromonas* spp. isolated from patients with diarrhoea. *Journal of Clinical Microbiology*, **25**, 2044-2048.

Nagy, L.A. and Olson, B.H. (1982) The occurrence of filamentous fungi in drinking water distribution systems. *Canadian Journal of Microbiology*, **28**, 667-671.

Nagy, L.A. and Olson, B.H. (1985) Occurrence and significance of bacteria, fungi and

yeasts associated with distribution pipe surfaces. Proceedings, AWWA, WQTC, Houston, Texas, December.

Newson, S.W.B. (1968) Hospital infection from contaminated ice. *Lancet ii*, 620.

Niemi, R.M., Knuth, S., Lundstrom, K. (1982). Actinomycetes and fungi in surface waters and in potable water. *Applied and Environmental Microbiology*, **43**, 378-388.

Olivieri, V.P., Bahalian, A.E., Bossung, K.W. and Lowther, E.D. (1985) Recurrent coliforms in water distribution systems in the presence of free residual chlorine. In *Water Chlorination, Chemistry, Environmental Impact and Health Effects.* ed. Jolley, R.L., Bull, R.J., Paris, W.P., Katz, S., Roberts, M.M. and Jcubs, V.A., Michigen: Lewis Publishers Inc.

Olson, B.H. (1982). Assessment and implication of bacterial regrowth in water distribution systems. EPA-60/S2-82-072.

Olson, B.H., Ridgeway, H.F. and Mears, E.G. (1981). Bacterial colonization of mortar-lined and galvanized-iron water distribution mains. Proceedings, AWWA Annual Conference, St. Louis, June 1981.

Reasoner, D.J. and Geldreich, E.E. (1985). A new medium for the enumeration and subculture of bacteria from potable water. *Applied and Environmental Microbiology*, **49**, 1-7.

Reilly, J.K. and Kippin, J.S. (1983) Relationship of bacterial counts with turbidity and free chlorine in two distribution systems. *Journal of the American Water Works Association*, **75**, 309-312.

Ridgeway, H.F. and Olson, B.H. (1981) Scanning electron microscope evidence for bacterial colonization of a drinking water distribution system. *Applied and Environmental Microbiology*, **41**, 274-287.

Ridgeway, H.F. and Olson, B.H. (1982) Chlorine resistance patterns of bacteria from two drinking water systems. *Applied and Environmental Microbiology*, **44**, 972-987.

Rosenzweig, W.D. (1987) Influence of phosphate corrosion control compounds on bacterial growth. EPA Project Summary 600/S2-87/045.

Rosenzweig, W.D. and Pipes, W.O. (1988). Fungi from potable water. Interaction with chlorine and engineering effects. *Water Science and Technology*, **20**, 153-159.

Rosner, R. (1964) *Aeromonas hydrophila* as the etiological agent in a case of some gastroenteritis. *American Journal of Clinical Pathology* , **42**, 402-404.

Schoenen, D. and Schöler, M. (1985) *Drinking water materials: Field observation and Methods of Investigation.* Chichester, England, Ellis Horwood Limited.

Schofield, G.M. and Locci, R. (1985) Colonization of component of a model hot water system by *Legionella pneumophila. Journal of Applied Bacteriology*, **58**, 151-162.

Seidler, R.J., Morrow, J.E. and Bagley, S.T. (1977) Klebsielleae in drinking water emanating from redwood tanks. *Applied and Environmental Microbiology*, **33**, 893-900.

Stanley, J.T. (1968) *Prosthecomicrobium* and *Ancalomicrobium*; new prothecate freshwater bacteria. *Journal of Bacteriology*, **95**, 1921- 1942.

Stotzky, G. (1967). Clay mineral and microbial ecology. *Trans. N.Y. Acad. Sci.*, **30**, 11-21.

Tamper, J. (1987). Immobilisatie van micro-organen en toepassingen in de milieuhygiene. *H_2O*, **20**, 375-377.

Taylor, W. (1947) Bacteriology of water samples from water mains. *Journal of Applied Bacteriology* ,**10**, 34-58.

Touvinen, O.H., Button, K.S., Tuovinen, A., Carlson, L., Mair, D.M. and Yut, A.L. (1980) Bacterial, chemical and mineralogical characteristics of tubercles in distribution pipe lines. *Journal of the American Water Works Association*, **72**, 626.

Touvinen, O.H. and Hsu, J.S. (1982). Aerobic and anaerobic microorganisms in tubercles from Columbus, Ohio water distribution systems. *Applied and Environmental Microbiology*, **44** 761.

Van der Kooij, P. and Zoetemann, B.C.J. (1978). Water quality in distribution systems. International Water Supply Association Congress. Kyoto, Special Subject 5.

Van der Kooij, D., Oranje, J.P. and Hijnen, W.A.M. (1982a) Growth of *Pseudomonas aeruginosa* in tap water in relation to utilization of substrates at concentration of a few micrograms per litre. *Applied and Environmental Microbiology*, **44**, 1086-1096.

Van der Kooij, D., Visser, A. and Hijnen, W.A.M. (1982b). Determining the concentration of easily assimilated organic carbon in drinking water. *Journal of the American Water Works Association*, **74**, 540-545.

Van der Kooij, D. and Hijnen, W.A.M. (1988) Multiplication of *Klebsiella pneumoniae* strain in water - a low concentration of substrates. *Water Science and Technology*, **20**, 117-123.

Victoreen, H.T. (1977). Water quality deterioration in pipeline. In *Proceeding of the Water Quality Technical Conference*, Kensas City, MO, AWWA, Denver.

Ward, N.R., Wolfe, R.L., Mean, E.G. and Olson, B.H. (1982) The inactivation of total and selected gram-negative bacteria by inorganic monocloramine and dichloramine. In *Proceedings of the AWWA QTC*, Denver, pp.81-97.

Wolfe, R.S., Ward, N.R. and Olsen, B.H. (1985) Inactivation of heterotrophic bacterial population in finished drinking water by chlorine and chloramine. *Water Research*, **19**, 1393-1403.