PREVALENCE AND SURVIVAL OF *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA* SPP. IN SURFACE WATERS OF SOUTHERN ALBERTA

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Dedication

This work is dedicated to Drs Eric Norris, Peter Schuepp, Mansel Griffiths, and Dan Johnson, who demonstrated the importance of asking questions of value and questioning what is presented as the truth, and to Murray Johnson who helped me to realize that discovery can be a creative process. This is also dedicated to the memories of Robin Costain and Carl Nishi.

Abstract

E. coli O157:H7 were isolated from 0.86% (n=1520) water samples and *Salmonella* species from 6.04% (n=1456) samples collected within the Oldman River watershed in southern Alberta. Peak prevalence of *E. coli* O157:H7 in July 2000 was 6.3% (n =48). Peak prevalence of *Salmonella* was 16.2% (n =11) in August 1999 and 33.% (n=42) in July 2000. Prevalence was greater in water from some sampling locations than from others. In non-filtered surface water *E. coli* O157:H7 and *S. typhimurium* numbers decreased significantly faster at 20 °C than at 10 °C (P =0.000); however this difference did not exist when the same water was filtered (P=0.439). Pathogen survival in one water sample was greater when it was filtered (0.2μm pore) than when it was not filtered even though there were no autochthonous bacteria in the water prior to filtration.

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Table of Contents

List of Tables

List of Figures

1. Literature Review: Prevalence and Survival of *Escherichia coli* O157:H7 and *Salmonella* spp. in Surface Waters of Southern Alberta

1.1 Introduction

Although enteric pathogens *E. coli* O157:H7 and *Salmonella* are commonly associated with contaminated foods (Doyle, *et al.* 1997; D' Aoust, 1997), waterborne outbreaks of enteric disease caused by these pathogens have also caused morbidity and mortality worldwide (Kondro, 2000; Barwick, *et al.*, 2000; Swerdlow, *et al.* 1992; Akashi *et al.*, 1994; Koplan, 1978). In order to better understand the risk of exposure of individuals or a population to pathogens through contaminated water supplies, prevalence data of pathogens in water, and models of their survival in water are required (Neumann and Foran, 1997; Buchanan and Whiting, 1996; Marks *et al.*, 1998).

There has been very little published on the prevalence of *E. coli* O157:H7 in surface water (McGowan *et al.*, 1989). Prevalence of *Salmonella* has been studied in the context of its relationship to indicator organisms in surface waters of Australia (Townsend, 1992), Spain (Polo *et al.*, 1998), Greece (Arvanitidou *et al.*, 1997; Efstratiou *et al.*, 1998), and Italy (Luppi, *et al.* 1988). In the present study the prevalence of *E. coli* O157:H7 and *Salmonella* spp in surface water was estimated based on samples from sites within the Oldman River basin in southern Alberta.

Governmental agencies have used microbial risk assessments of water based on indicator organisms, such as faecal coliforms and *E. coli* bacteria, as a basis for establishing maximum allowable contamination level limits for water (Macler and Regli, 1993). However, the relationship between indicator organism counts and the presence of pathogens in water is variable among studies (Polo *et al.*, 1998; Arvanitidou *et al.*, 1997; Townsend, 1992; Luppi, *et al.* 1988; Efstratiou *et al.*, 1998). In this study associations between indicator organism concentrations and the presence of *E. coli* O157:H7 and *Salmonella* spp. in the water samples were examined in this study. As well, comparisons were made between the rating of water samples in terms of the microbial water quality guidelines used in Alberta and the presence of *E. coli* O157:H7 and *Salmonella* spp.

Survival of pathogenic bacteria in water is influenced to varying degrees by water temperature, species of pathogen, and autochthonous bacteria in water (Flint, 1987; McCambridge and McMeekin, 1980; Wang and Doyle, 1998; Rhodes and Kator, 1988; McCambridge and McMeekin, 1981; Warburton *et al.*, 1994). The present study also examines the survival of *E. coli* O157:H7 and *Salmonella typhimurium* in surface water samples obtained within the Oldman River watershed and assess the effects of water temperature, species of pathogen, autochthonous bacteria, inoculum concentration, sampling site, and filtration on various survival characteristics.

1.1.1 Microbiology of *E. coli* O157:H7

E. coli are facultative anaerobic Gram-negative rod-shaped bacteria in the family *Enterobacteriaceae*. *E. coli* serogroups are classified based on surface lipopolysaccharide antigens. Presently within the species *E. coli* there are 174 O antigens and 56 H antigens identified (Doyle, *et al.*, 1997). Many *E. coli* have only been isolated from the faeces of healthy individuals. However, others are associated with disease in humans (Hull, 1997). *E. coli* possessing O-antigens O157, O26, O103, O104, and O111 are designated as enterohaemorrhagic *E. coli* (EHEC); strains within these serogroups are responsible for haemorrhagic colitis (HC) (Hull, 1997; Doyle *et al.*, 1997). *E. coli* O157:H7 and *E. coli*

O157:NM (non-motile) strains are the strains most frequently associated with outbreaks of HC and with haemolytic uraemic syndrome (HUS) (Griffin and Tauxe, 1991; Willshaw *et al*, 1997). Characteristics of HUS include anemia, thrombocytopaenia, and acute renal insufficiency (Tarr, 1994; Doyle *et al.*, 1997). HUS is the leading cause of acute renal failure in children (Doyle, *et al.*, 1997). It has been estimated that 10% of children with symptomatic infection with *E. coli* O157:H7 develop HUS.

Two properties of *E. coli* O157:H7 presently identified as major virulence factors include the ability to, 1) adhere to mucosal surfaces, and 2) produce verotoxins (Griffin, 1995).

1.1.1.1 Histopathology

The process by which EHEC become attached to host cells requires a pathogenicity island found within their genome called the locus of enterocyte effacement (LEE) (Nataro and Kaper, 1998). This region of the genome contains a family of genes encoding for proteins called Esps, (enteropathogenic *E. coli* secreted proteins), as well as intimin and its translocated intimin receptor (Tir) (Vallance and Finlay, 2000). In EHEC, EspA, EspB, intimin, and Tir appear to be factors in the formation of attaching and effacing (AE) lesions (Li *et al*, 2000; Nataro and Kaper, 1998; Tatsuno *et al.*, 2000). EspA, EspB, and Tir are translocated into the host cell by a type III secretion system (Nataro and Kaper, 1998; DeVinney *et al.*, 1999). It has been suggested that EspA allows for the initial diffuse adherence of EHEC to epithelial cells (Tatsuno *et al.*, 2000). Once Tir is within the host cell, intimin, a 94-kDa outer membrane protein, encoded by the *eae* gene (Willshaw, *et al.* 1997), binds to Tir, allowing intimate attachment of the pathogen to the host cell. This induces the formation of microcolonies of bacteria on the surface of epithelial cells lining the intestine (Tatsuno *et al.*, 2000). Following intimate attachment of the pathogen to intestinal cells an accumulation of filamentous actin within intestinal cells beneath the attachment site results in a pedestal formation on which EHEC rest (Donnenberg and Kaper, 1992).

Although the mechanisms that trigger the diarrheoa response are uncertain, one model proposes that AE lesions in epithelial cells of the large intestine caused by *E. coli* O157:H7 result in thinning of the microvilli (Doyle *et al.*, 1997). As a result, there is a substantial decrease in the adsorptive capacity of the microvilli. Thus fluids are prevented from being adsorbed and instead, are secreted from the intestine (Nataro and Kaper, 1998).

1.1.1.2 Verotoxins

In a study by Konowalchuk *et al* (1977) 10 of 136 *E. coli* strains tested were classified as verotoxin (VT) -producing *E. coli* due to the cytotoxic effect of culture supernatants on Vero cells. Of the 10 strains that induced this particular cytotoxic effect, 7 were associated with diarrhoea in human infants.

EHEC produce two classes of antigenically distinct Shiga-like toxins, VT1 and VT2 (Scotland *et al.*, 1985). It has been suggested that the ability of EHEC to produce VTs that play an essential role in the development of bloody diarrhoea and hemorrhagic colitis (Nataro and Kaper, 1998). Genes coding for VT1 and VT2 toxins in some VTEC strains are encoded by morphologically distinct bacteriophages (Willshaw *et al*, 1987; O'Brien *et al*, 1984).

1.1.2 Epidemiology of *E. coli* O157:H7

In the U.S, food transmits more disease caused by *E. coli* O157:H7 than does water. From 1988 to 1997, this organism caused 4664 food borne-disease cases (mean = 466.4 cases annually), resulting in 95 outbreaks and 8 deaths (Bean *et al.*, 1996; Olsen *et al.*, 2000). The United States Department of Agriculture (USDA) reported that 14% of outbreaks caused by *E. coli* O157:H7 were associated with contaminated ground beef; 31% were attributed to spread from person to person, and in 28% of cases the route of transmission was unknown (USDA, 1997).

In the United States, from 1993 to 1998, the Centers for Disease Control (CDC) and the U.S. Environmental Protection Agency (EPA) (Barwick, *et al.*, 2000; Kramer *et al.* 1996; Levy, *et al.* 1998) reported that *E. coli* O157:H7 caused 257 cases of gastrointestinal disease in individuals using recreational surface water, 197 cases of drinking water-related disease, and 1 death associated with an outbreak at a water park. Between 1993 and 1998, an annual mean of 75.7 cases of waterborne disease caused by *E. coli* O157:H7 were reported to these agencies.

Beach water has been associated with disease outbreaks caused by *E. coli* O157:H7 in Illinois (Warmer *et al.*, 1996) and Oregon (Keene *et al.* 1994). In the Illinois outbreak leakage from outdoor toilet facilities, proximity to cattle farms and sewage outlets were ruled out as possible sources of contamination. Keene *et al.* (1994) also could not find any extraneous source of contamination in an outbreak in Oregon, and suggested that children who were not toilet-trained could have been the source.

Infection from drinking water, either from well water or tap water has also been reported. Sewage contamination of a water distribution system was considered the source of an outbreak of *E. coli* O157:H7 infection in Missouri, in 1989, in which 243 individuals became ill, 2 developed HUS, and 4 died (Swerdlow *et al.*, 1992). Akashi, *et al.* (1994) also reported that 174 children became ill; of these 42 were culture-confirmed as being infected with *E. coli* O157:H7 and 14 developed HUS from drinking contaminated tap water at a school in Japan that was drawn from a well. Six hundred and thirty-three cases of illness caused by *Campylobacter* and *E. coli* O157:H7 were reported in Fife, Scotland in 1995, following contamination of a public water system by treated sewage (Jones and Roworth, 1996). During this outbreak 2 people developed HUS. In 1999, in New York State, 116 individuals were infected by drinking *E. coli* O157:H7-contaminated beverages prepared with unchlorinated well water, following which, 11 individuals developed HUS, and 2 died (Anonymous, 1999). During an outbreak of *E. coli* O157:H7 infection in Walkerton, Ontario in 2000, due to a contaminated water supply and problems with chlorination treatment, more than 700 individuals became ill, and 7 deaths were attributed to the outbreak (Kondro, 2000).

The small amount of water assumed to have been ingested by infected swimmers during swimming-related outbreaks suggests that the infectious dose for *E.coli* O157:H7 is low (Keene et al, 1994). A low infectious dose may also be inferred by the ease of person-to-person transmission (Boyce et al, 1995).

In a study of HUS in children less than 15 years of age reported to Canadian hospitals in cities with populations over 350,000 from 1986 to 1988 (226 patients), the national incidence was 1.44 cases / 100,000 individuals. In Alberta and Ontario the incidence was 2.78 and 0.97/100,000, respectively (Rowe, 1991). Waters *et al.* (1994) found that during part of the 1980s and 1990s Alberta had the highest rate of *E. coli* O157:H7 infections in

North America and that health units in and near Calgary had a higher rate than did those north of Calgary. They suggested possible reasons for this phenomena were more intense clinical screening for VTEC within southern Alberta, greater surface water contamination, more intense cattle farming in the region and a greater associated probability of contamination of water sources with *E. coli* O157:H7, more frequent consumption of contaminated water in the south than in the north of the province, but noted there are no data to substantiate these hypotheses.

1.1.3 Reservoirs of *E. coli* O157:H7

Prevalence of *E. coli* O157:H7 in cattle faeces appears to vary with detection method, age of animal, and time of year. *E. coli* O157:H7 was detected by plating on sorbitol MacConkey agar with cefixime and tellurite (ctSMAC) in the faeces of 4.0% of dairy calves 2 to 8 months of age (*n* = 351) (Sanderson *et al*, 1995), and 1.5% of dairy calves 1 to 6 months of age (*n* =1515) (Hancock *et al.*, 1997). In other studies *E. coli* O157:H7 was detected in the faeces of 16.1% of dairy cattle (*n*= 1661), and 13.4% of beef cattle after slaughter (*n*= 1840) in England (Chapman *et al.*, 1997), and in the faeces of 1.9% of cattle in a cow-calf farm (*n*=2058)(Sargeant, *et al.*, 2000) using immunomagnetic separation followed by plating on ctSMAC. In another study, the pathogen was detected in faeces of 2.9% of calves from 1 day to 4 months of age (*n*=662) when using sorbitol MacConkey agar (Zhao, *et al.*, 1995). Hancock, *et al.* (1997) reported 0.4% of adult cattle, 1.8% of weaned, and 0.9% unweaned heifers tested shed *E. coli* O157:H7. A review of 18 prevalence studies by Armstrong *et al.* (1996) suggested calves are more likely to shed these bacteria than are older cattle. Shedding of this organism from cattle appears to be seasonal. Over a 2-year period, Hancock, *et al.* (1997) observed a higher prevalence of *E. coli* O157:H7 in bovine faeces in 14 cattle herds during summer months than in the rest of the year, peaking at 2.6% in June.

E. coli O157:H7 are zoonotic bacteria that has been isolated from healthy or ill cattle as well as from sheep, pigs, deer, and insects (USDA, 1997; Armstrong *et al.*, 1996; Zhao *et al.*, 1995; Cornick *et al.*, 2000; Chapman *et al.*, 1997; Sargeant *et al.*, 1999; Shere *et al.*, 1998). It appears that poultry and wild birds are not a common reservoir of *E. coli* O157:H7 (Chapman *et al.*, 1997; Doyle *et al.*, 1997; Adensiyun, 1999; Armstrong *et al.*, 1996).

1.1.4 Microbiology of *Salmonella*

Salmonella are facultative anaerobic Gram-negative rod-shaped bacteria of the family Enterobacteriaceae. They typically are motile with peritrichous flagella. Most do not ferment lactose, although many strains of *S. arizonae* do (Le Minor, 1986). Most *Salmonella* produce gas from glucose, and produce hydrogen sulfide gas and are urease-negative (Le Minor, 1986). The diversity in reactions in biochemical tests in *Salmonella* species has led some workers to question their use in species identification (D'Aoust, 1997). While there are over 2200 serotypes of this genus, many *Salmonella* species do not commonly cause infection in humans, and those that do, are often isolated from livestock, wild animals and birds (Baird-Parker, 1990).

Infection by *Salmonella* occurs when they invade the intestinal tissues and lymph nodes (Finlay, 1994). When the pathogen is very close to the intestinal epithelium, microvilli of the epithelial cells elongate and the cytoplasm of the cells nearest the surface begin to distort (Finlay, 1994). Attachment of *Salmonella* cells to the epithelial cells also involves both fimbriated and non-fimbriated surface adhesins (Polotsky *et al.*

1994). Following signal exchange the pathogen is internalised into the epithelial cell. (Polotsky *et al.* 1994). *S. typhimurium* has been shown to produce low levels of a cholera-like enterotoxin (Prasad, *et al,* 1990). The mechanisms by which these toxins cause diarrhea are not clear (Finlay, 1994). Prasad *et al.* (1990) demonstrated that crude lysates of *S. typhimurium* Q1 possesed increased levels of cyclic adenosine monophosphate (cAMP) in intestinal epithelial cells. cAMP, a factor in diarrhoeal disease caused by cholera toxin, inhibits the absorption of Na^+ and Cl^-, and stimulates the secretion of these ions (Adams and Moss, 1995). The osmotic imbalance results in a transfer of electrolytes, which produces a flow of water in excess of the absorptive capacity of the intestine (Adams and Moss, 1995). Diarrhea caused by *Salmonella* infection has been shown to be associated with increased infiltration of white blood cells into the infected tissue. In association with this inflammation, Goblet cells from the small intestinal epithelial lining are stimulated to secrete mucous by activation of an enzyme that causes large amounts of fluids to be produced (Polotsky *et al.* 1994).

1.1.5 Epidemiology of *Salmonella*

Infection by *Salmonella* in humans may result in gastroenteritis, septicemia, diarrhea, fever, and vomiting (Moe, 1997; Le Minor, 1986). Septicemia following infection by *S. typhi* can be accompanied by fever, nausea, and delirium (Baird-Parker, 1990). The average mortality rate of untreated typhoid fever is 10% (Atlas, 1997). Worldwide, one of the principal causes of bacterial food borne disease is *Salmonella* (D'Aoust, 1997). CDC and US EPA reported 906 of 5174 (17.5%) food borne-disease cases and 51 of 98 (52.0%) foodborne-disease deaths were caused by *Salmonella* (Bean *et al.*, 1996; Olsen *et al.*, 2000). The spread of infection is principally due to poor hygiene in preparation of contaminated foodstuffs of animal origin (Kaplan, 1997). Of all etiologic agents of food borne disease, *S. enteriditis* caused the greatest number of mortalities in the U.S. from 1994 to 1997 (Olsen *et al.*, 2000). This serotype is most commonly linked to poultry (Kaplan, 1997). Eggs and egg-based products, dairy products, chocolate, fruits, and vegetables have also been identified as vehicles of major foodborne outbreaks of human salmonellosis (D'Aoust, 1997). *Salmonella* is not frequently identified as the cause of waterborne disease outbreaks. Of the 70 outbreaks of waterborne gastroenteritis reported to the CDC and the U.S. EPA from 1993 to1998, only 2 were caused by *Salmonella* (Barwick, *et al.*, 2000; Kramer *et al.* 1996; Levy, *et al.* 1998). However, the outbreak caused by *S. typhimurium* in 1993, most likely due to a water tower unprotected from wild-bird droppings, resulted in 625 illnesses and 7 deaths (Kramer, *et al.*, 1996).

A dose-response model of *S. typhosa* in humans developed by Holcomb *et al.* (1999) suggested the infectious doses for a predicted frequency of infection of 0.10 (1 out of 10 persons would become ill) was less than 10 cells. In a study of 12 outbreaks of typhoid fever, the estimated number of organisms ingested in 10 of the outbreaks was less than 10^3 cells (Blaser and Newman, 1982). Individuals infected during a waterborne outbreak of disease caused by *S. typhimurium* ingested an estimated average of 17 bacteria / litre of water (Blaser and Newman, 1982).

1.1.6 Reservoirs of *Salmonella*

Salmonella have been isolated from pigs, poultry, cattle, horses, seabirds, insects, various foodstuffs, and humans (Baird-Parker, 1990; Uyttendaele, *et al.* 1999; Oosterom, *et al.* 1985; Higgins *et al.*, 1982; Van Donkersgoed, *et al.* 1999; Galland *et al.*, 2000). In a study of prevalence in raw chicken broilers, *Salmonella* was isolated from 25.9%

samples (7 of 27)(Chang, 2000). Sources or animal reservoirs of *Salmonella* are listed in Table 1.1.

Various sources have been associated with waterborne outbreaks of salmonellosis. Koplan *et al.* (1978) proposed rainwater used for cooking that had been contaminated by wild birds, was the source of contamination in a *Salmonella* disease outbreak in Trinidad. Wild birds were also associated with a *S. typhimurium* –related outbreak in Missouri, in which 625 people became ill and 7 died (Kramer *et al.* 1996). In New York State, in 1996, well water contaminated by runoff from nearby farms was suggested as the source of infections in an outbreak of salmonellosis associated with *S. hartford* (Van Houten *et al.*, 1996).

Table 1-1. *Salmonella* prevalence in animal reservoirs. (A) indicates selective enrichment followed by selective plating; (B), resuscitation in buffered peptone water followed by selective plating; (C), selective enrichment, selective plating and enzyme linked immunosorbent assay.

Reservoir	Samples tested	Isolation method	Prevalence, % (*n*)	Author (s) (year)
Poultry	Faeces	A	38.0 (1453)	Higgins, *et al.*(1982)
	Whole broilers	A	25.9 (27)	Chang (2000)
Pigs	Intestinal contents, lymph nodes	B	24.3 (210)	Oosterom *et al.* (1985)
	Rectal contents, lymph nodes	A	22.3 (7756)	Edel *et al.* (1976)
Cattle	Rectal contents	C	40 (120)	Galland, *et al.* (2000)
Seagulls	Droppings	A	26.7 (60)	Edel *et al.* (1976)

1.2 Waterborne Transmission of Enteric Bacteria

Dispersal of infectious agents via water systems has been documented as far back as 1854, when John Snow determined that an outbreak of cholera in London was due to a contaminated public water supply (Stolley and Lasky, 1998). *Vibrio cholerae* and *Salmonella typhi*, from the family Enterobacteriacae, have caused a multitude of death and illness due to waterborne infection worldwide for centuries, an indication of the considerable human health risk associated with water contaminated by members of this family of bacteria (Moe, 1997). While the protozoan *Cryptosporidium parvum* has been the leading cause of gastroenteritis from drinking and recreational water in the United States from 1993-1998, the bacterial enteric agents such as *E. coli* O157:H7, which caused 454 cases of gastric illness from 1993 to1998, and *Salmonella* species, such as *S. typhimurium*, which infected 625 individuals and caused 7 deaths, are important with respect to morbidity and mortality associated with water (Kramer *et al.*, 1996; Levy *et al.*, 1998; Barwick *et al.* 2000). It has been suggested that waterborne transmission favoured the evolution of highly virulent pathogens such as *V. cholerae, Shigella dysenteriae,* and *S. typhi,* which are largely associated with waterborne infection. Ewald *et al.* (1998) proposed that pathogens that are transmitted by living hosts benefit when the infection they cause does not kill the host, and consequently evolve low virulence. By contrast, waterborne pathogens, which do not require an infected host as a vehicle of transmission, retain, relative to pathogens that rely on living hosts for transmission, a higher level of virulence. This hypothesis was developed as a possible explanation for the increasing trend in deaths per infection with increase in percent of outbreaks involving waterborne transmission for various pathogens (Ewald *et al.*, 1998).

1.3 Prevalence in Surface Water

McGowan *et al.* (1989) isolated *E. coli* O157:H7 from 1 of 30 water samples from 15 locations in urban and rural regions of Philadelphia. This is the only study in the literature not associated with a waterborne disease outbreak caused by this organism. Other studies of the presence of *E. coli* O157:H7 in water have been performed following outbreaks of disease. Water samples of the contaminated water distribution system that was associated with *E. coli* O157:H7 infections in Missouri in 1989 were tested for the presence of *E. coli* O157:H7 but the pathogen was not detected (Swerdlow *et al.*, 1992). *E. coli* O157:H7 was also isolated in water implicated in an outbreak associated with well water by PCR assays, but prevalence was not reported (Anonymous, 1999).

The prevalence of *Salmonella* in surface water has been reported in many studies (Townsend, 1992; Arvanitidou *et al.*, 1997; Polo *et al*, 1998; Efstratiou *et al.*, 1998; Luppi, *et al*, 1988). Rates of *Salmonella* prevalence are highly variable among surface water studies, which vary in culture methods used and type of water sampled (e.g. estuarine, sea, lake, river)(Table 1-2). Prevalence can be high in studies of locations with both high (Sharma and Rajput, 1996) and low (Townsend, 1992) anthropological inputs.

Table 1-2. Studies of *Salmonella* prevalence in surface water. *Salmonella* strains were isolated using selective enrichment and selective plating on various media. Prevalence varies among types of reservoirs and among studies.

Number of samples, sites, years	Reservoir (Country)	Prevalence, %	Citation
75, 8, 3	river (Spain)	58.7	Polo *et al.*, (1998)
2237, 213, 3	sea water (Spain)	5.9	
128, 14, 3	freshwater reservoir (Spain)	14.8	
44, 1, 1	lake (Greece)	4.5	Arvanitidou *et al.*, (1997)
84, 4, 1	river (Greece)	7.1	
45, 1, 2	tropical pond, 4 km^2 basin (Australia)	69	Townsend, (1992)
45, 1, 2	tropical pond, 130 km^2 basin (Australia)	96	
80, NA, 3	canal (Italy)	25.0	Luppi, *et al.* (1988)
303, 21, 2	sea water (Greece)	25.7	Efstratiou *et al.*, (1998)

1.4 Pathogenic Bacteria and Indicator Bacteria

In a study conducted in northern Greece, Arvanitidou *et al.* (1997) found no significant difference using non-parametric tests, between concentrations of faecal coliforms, total coliforms, or faecal streptococci in samples with *Salmonella* and those without. In another study in Greece conducted by Efstratiou *et al.* (1998), only total coliform counts were significantly correlated (logistic regression) with the presence of *Salmonella*, while faecal coliforms and faecal streptococci were not. As well, it was determined that while total coliform counts were correlated with *Salmonella* presence in moderately polluted waters, they were not correlated in highly polluted water. Indeed, of the three indicator groups, total coliform counts had the weakest correlation with *Salmonella* presence in highly polluted water. In two tropical ponds with no apparent human or domestic animal faecal contamination, total and faecal coliforms, and enterococci were correlated (non-parametric tests) with the presence of *Salmonella* (Townsend, 1992). Of the two ponds, higher correlation coefficients were noted in samples from the pond with the smaller watershed. In a study conducted by Polo *et al.* (1998) in Spain, geometric means of total coliform, and faecal streptococci counts were higher (non-parametric tests) for *Salmonella*-positive than for *Salmonella* - negative samples of river water, but that trend was reversed in samples of seawater. Among and within studies, then, differences exists in the correlation between indicator concentrations and the presence of *Salmonella*. There are no published studies of the relationship between *E. coli* O157:H7 and indicator organisms.

It has been recommended by Health Canada (Federal-Provincial Subcommittee on Recreational Water Quality, 1992) and the American Public Health Association

(American Public Health Association, 1985) that routine monitoring of microbial water quality involve tests for coliform concentrations rather than for the presence of specific enteric pathogens, as there is no test available that can isolate all pathogens and the absence of a given pathogen does not preclude the presence of others.

Faecal coliform counts enumerate Gram-negative, lactose-fermenting bacteria, and *E. coli* is one of many species that fall into the faecal coliform category. Faecal coliforms and *E. coli* are used to indicate faecal contamination of water (Atlas, 1997). The Surface Water Quality Guidelines for use in Alberta state that the limit for *E. coli* and faecal coliform bacteria in recreational water should be no greater than 200 counts/ 100 ml as a geometric mean of at least 5 samples taken within 30 days. Re-sampling is required if any sample has a level greater than 400 *E. coli* /100 ml (Natural Resources Service, Water Management Division, Alberta Environment, 1999). The maximum allowable concentration for coliforms in drinking water is zero, as established in the Summary of Guidelines for Canadian Drinking Water Quality (Federal-Provincial Subcommittee on Drinking Water, 1999), although if there are no *E. coli* in a sample, up to 10 total coliforms are considered acceptable. The 1999 Canadian Water Quality Guidelines for the Protection of Agricultural Water Use recommends the guideline for irrigation water to be 1000 total coliforms /100 ml and 100 faecal coliforms /100 ml (Canadian Council of Ministers of the Environment, 1999). Table 1-3 lists the various water use guidelines applicable in Alberta.

Table 1-3 Guidelines for microbial water quality for use in Alberta

Water use	Citation	Guideline or maximum limit
Drinking	Federal-Provincial Subcommittee on Drinking Water (1999)	10 total coliforms/100 ml and 0 *E. coli* / 100 ml
Recreation	Natural Resources Service, Water Management Division, Alberta Environment (1999)	400 *E. coli* / 100 ml
Irrigation	Canadian Council of Ministers of the Environment (1999)	100 faecal coliforms/100 ml and 1000 total coliforms/100 ml

1.5 Survival of Enteric Bacteria in Water

The survival and regrowth of bacteria in public water supplies is of great concern to the water treatment industry (LeChevalier *et al.*, 1996). As well, the extended duration of bacterial survival in drinking water (>95 days) has recently been recognised (Warburton *et al.*, 1994; Byrd *et al.* 1991). Outbreaks of waterborne disease caused by enteric bacteria such as *E. coli* O157:H7 and *Salmonella* suggest they have an ability to survive in sub-optimal water environments. Environmental conditions may be factors in the degree to which these bacteria can survive, and consequently, may influence the risk to human health associated with waterborne pathogenic bacteria.

Laboratory and *in-situ* surface water survival studies (Rice and Johnson, 2000; Rhodes and Kator, 1988; McCambridge and McMeekin, 1980; McCambridge and McMeekin, 1981; Pommepuy, *et al.* 1996; Pokorny, 1988; Flint, 1987; Wang and Doyle, 1998; Chao *et al*, 1988) have been conducted to examine the influence on survival exerted by factors such as competition with and predation by other indigenous micro-organisms, water temperature, species of pathogen, solar radiation, chemical oxygen demand, antibiotic resistance of the pathogens, and toxic materials.

1.5.1 Difference in Response Based on Species

There appears to be no consistent difference between the ability of *E. coli* and *Salmonella* to respond to stress in water. Rhodes and Kator (1988) observed that declines in populations of *E. coli* were more rapid than those of *S. tennessee* and *S. typhimurium* in filtered water at temperatures below 10^0C, although this difference did not exist in non-filtered water. In tropical freshwater the percentage of active cells and the frequency of cell division were greater for *E. coli* than for *S. typhimurium* after 24 hours, but after 108 hours direct counts of both species were not significantly different (Jimenez *et al.* 1989).

1.5.2 Effect of Temperature

Temperature is one of the most influential factors on bacterial growth rate (Atlas, 1997). Pokorny (1988) noted in his study of *S. enteritidis* that survival time in water held at 4 °C was double that in water at 20 °C.

Furthermore, it appears that indigenous bacteria in water have an interactive effect with temperature on pathogen survival. Wang and Doyle (1998) observed that at 8 °C and 25 °C the concentration of *E. coli* O157:H7 strains in filtered tap water decreased at rates of 2.4 CFU/ml/week and 76.9 CFU/ml/week , respectively. In the same study, *E. coli* O157:H7 concentration decreased in lake water at a rate of by 7.7 CFU/ml/week at 8 °C, and 333.3 CFU/ml/week at 25 °C. Thus, in both water samples the average rate of decrease in *E. coli* O157:H7 concentration was more rapid at 25 °C than at 8 °C. The difference between the average rate of decrease at 8 °C and that at 25 °C, i.e. the effect of temperature on survival, was greater when indigenous bacteria were present in lake water (325.6 CFU/ml/day) than when they were absent in filtered water (74.5 CFU/ml/day).

Flint (1987) observed the concentration of *E. coli* K12 in filtered water (0.45 μm pore) decreased at an average rate of 40 CFU/ml/day at 15 °C and 71.4 CFU/ml/day at 25 °C. The same bacteria in non-filtered water decreased at a rate of 100.0 CFU/ml/day at 15 °C and 125.0 CFU/ml/day at 25 °C. Hence, the average rate of decrease was greater (i.e., survival was less) at 25 °C than at 15 °C, which indicates a trend similar to that presented in the studies of Pokorny (1988) and Wang and Doyle (1998). However, in contrast to the study (Wang and Doyle, 1998), the difference between rate of decrease at low and high temperatures observed by Flint (1987) was greater when indigenous bacteria were absent (31.4 CFU/ml/day) than when they were present (25 CFU/ml/day).

1.5.3 Effect of Autochthonous Micro-organisms

Microbial populations influence survival of pathogens, by antagonistic (one species benefits, while the other declines), or mutualistic (both species benefit) interactions. Predation by protozoa, competition from other bacteria, and bacteriophages may lead to population decline (Chao *et al.*, 1988; Wang and Doyle, 1998), while survival within protozoa may provide protection from inhospitable environments (King *et al.*, 1988; Michel *et al.*, 1998). Growth of rod-shaped pathogenic bacteria (presumably *Legionella*) has been observed within *Acanthamoeba* species isolated from drinking water (Michel *et al.*, 1998). With this bacterial species, it is thought that multiplication within the amoeba host cell progresses until it is filled with the bacteria, after which the host is ruptured and the bacteria released are ingested by other amoebae.

Nine species of enterobacteriaceae, including *E. coli* and *S. typhimurium*, *Legionella*, and *Campylobacter* were able to survive in chlorine-treated water significantly longer when co-cultured with ciliated protozoans such as *Tetrahymena pyriformis*, and amoeba such as *Acanthamoeba castellanii* (King *et al.*, 1988) than in protozoa-free culture. Bacteria associated with the protozoa were able to resist digestion, survive in the nutrient-rich environment within the protozoa, and, simultaneously, survive exposure to levels of free available chlorine (a form of chlorine with a higher oxidation potential that is used in water treatment) well above the level required to kill bacteria. The authors proposed that the ability to survive digestion by protozoa might have evolved in pathogenic bacteria as a means of persisting in environments not conducive to survival.

Chao *et al.* (1988) estimated the effect of protozoa on the survival of *S. enteritidis* by comparing their survival in river water to that in the same water supplemented with cycloheximide, an inhibitor of protozoan protein synthesis. Within the first 4 days, plate counts of the bacteria decreased approximately 10 CFU/ml in water with cyclohexamide and 10^3 CFU/ml in water without cyclohexamide. Also, in water without cyclohexamide, a rapid decrease in the bacteria during the first 2 days coincided with a rapid increase in the level of protozoa. The authors concluded that predation was a critical factor in the survival of *S. enteritidis* in water.

Peaks in microflagellates, lytic phage, and heterotrophic bacteria densities in water corresponded to maximum decline in *Salmonella* and *E. coli* in an *in-situ* study by Rhodes and Kator (1988). Flint (1987) found that although filtration through 0.45µm pore filters removed most indigenous bacteria from water samples; this pore size allowed the inclusion of small bacteria, such as *Vibrio* species, and filtration increased the duration of survival of *E. coli* at 4°, 15°, 25°, and 37°C. Other studies have found pathogen survival in water with a low concentration of indigenous predators was not greater than in filtered water (0.2µm pore size) (Santo Domingo *et al.*, 2000). Wang and Doyle (1998) examined survival of *E. coli* O157:H7 in water from 4 sources, (2 lakes, tap, and reservoir) each with a different concentration of total aerobic bacteria. They observed that pathogen survival rates decreased with increasing aerobic bacteria levels in water. During the course of the experiment, total aerobic bacteria counts declined 1 order of magnitude, while *E. coli* O157:H7 declined over 3 orders of magnitude. The authors concluded that this pathogen may not compete well against other bacteria.

Absence of predatory and competing organisms may allow prolonged survival of enteric bacteria; a cause for concern with respect to safety of bottled water and water distribution systems. Warburton *et al.* (1998) reported that a washed culture of *E. coli* O157:H7 (inoculation level of $\log_{10}$ 6.32) survived more than 247 days in spring water, while an unwashed culture (inoculation level of $\log_{10}$ 5.42) survived more than 309 days in sterile distilled water. When *E. coli* was introduced into a drinking water system that contained protozoa in both water phase and in a biofilm, the cells declined in numbers much more rapidly than they did when added to a system that had none, suggesting that the protozoa were feeding on the pathogenic bacteria (Sibille *et al.*, 1998). Other studies have indicated that synergies between two species of bacteria may exist and aid in the survival of enteric bacteria. For example, Warburton *et al.* (1994) observed greater survival of *Salmonella* species when co-inoculated with *Pseudomonas aeruginosa* in bottled water, from distilled, natural spring, and natural spring sources.

1.5.4 Effect of Nutrient Limitation

Enteric bacteria typically require high levels of nutrients, and hence, are more susceptible to the nutrient-limited conditions typical of most surface waters (McFeters, 1989). When essential nutrients, such as carbon, nitrogen, or phosphorus are unavailable, *E. coli* cells enter the stationary phase (Hengge-Aronis, 1993). Warburton *et al.* (1994) suggested that more prolonged survival of enteric bacteria in spring, mineral, and tap water than in distilled water might have been due to a higher nutrient content. Wang and Doyle (1998) found that the most rapid decline of *E. coli* O157:H7 occurred in water from a location with the highest level of total nitrogen, total inorganic carbon, total organic carbon, and aerobic bacteria. In this water, the high level of indigenous bacteria may have been supported by nutrients, but it is not clear if the high nutrient level in water is an advantage to both *E. coli* O157:H7 and indigenous bacteria, or if it favoured one population over the other. Rhodes and Kator (1988) found, in an *in-situ* study, that *E. coli* and *Salmonella* did not survive longer in surface water at a location with high levels of total phosphate and organic nitrogen than in one with relatively low levels of these compounds.

1.5.5 Effect of Other Factors

Solar radiation affects bacteria within the upper 30 cm of the water column, with minimal effects lower in the water column (Pommepuy *et al.* 1996). A decrease in concentration of culturable *E. coli* and *S. typhimurium* in estuarine water was observed with increasing cumulative exposure to sunlight (McCambridge and McMeekin, 1981). It is thought that pigments such as chlorophyll and flavonoids in surface water may be excited by solar radiation (between the 320 to 450 nm wavelength), resulting in the production of highly reactive oxygen species such as hydroxyl radicals and singlet oxygen that may be bacteriocidal (McGuigan *et al.*, 1999; Bahlaoui *et al.*, 1998).

Chemical oxygen demand (COD), a measure of organic loading of water, has been shown to affect pathogen survival in water. Pokorny (1988) observed that survival time of *Salmonella* was shorter in surface water with a high COD than in water with a lower COD. *Salmonella* survived (at $4^{O}C$) for 19 days in water with a COD concentration of 5.3 mg/l, and 2 days in water with a COD of 15.2 mg/l.

1.5.6 Microbial Stress Responses

Adaptation allows bacteria to survive effects of changing environmental conditions that are detrimental to their growth and reproduction (Abee and Wouters, 1999). During the early stage of a microbial stress response to a inhospitable environment, bacteria may change from their typical rod shape to a more spherical one, thereby allowing a lower rate of metabolism (Loewen *et al.* 1998).

Postgate (1976) proposed that bacteria, in a nutrient-limited state, can maintain themselves in a slow "cryptic" growth state, wherein lysed cells are metabolised by remaining active bacteria. In contrast to this model, Reeve *et al.* (1984) demonstrated that *E. coli* K12 did not exhibit cryptic growth in an environment of low exogenous levels of carbon. They observed no evidence of cell lysis during starvation. Furthermore, cellular material extruded into the media by non-viable cells did not promote growth in the remaining cells.

Kurath and Morita (1983) observed that 99% of the initial inoculum of *Pseudomonas* spp. in nutrient-poor media became non-viable within 2 days. The percent of glucose and glutamic acid that was released as CO_2 increased for 4 to 13 days, then decreased and stabilised after 36 days to a level similar to the initial. However, a small subpopulation (1% of initial population) of the inoculated culture survived for over 1 year. They concluded that cells that remained after 36 days had adapted to starvation by effectively shifting their metabolic balance from reproduction and biosynthesis to acquiring ATP for maintenance.

Bacterial RNA-polymerase is comprised of 3 core protein sub-units and a sigma factor sub-unit (Atlas, 1997). There have been many sigma factors identified and each facilitate the initial binding of core RNA polymerase to a specific promoter region during transcription from DNA to RNA (Abee and Wouters, 1999). Gene expression in *E. coli* and *S. typhimurium* under various stress conditions is regulated by the σ^S subunit of RNA polymerase, which is encoded by *rpoS* (Fischer *et al.,* 1998; Lee *et al.*, 1995; Hengge-Aronis, 1993; Loewen, *et al.*, 1998). Starvation is one stress that can result in inhibition of the rate of σ^S proteolysis, which, under non-stress conditions maintains the concentration of σ^S at low-levels in the cytoplasm of *E. coli* cells (Fischer *et al.*, 1998; Hengge-Aronis, 1993). The inhibition of σ^S proteolysis in nutrient-limiting conditions, may trigger nutrient-specific regulatory systems in *E. coli* to enable more efficient nutrient uptake (Hengge-Aronis, 1993). Under aerobic, phosphate-starved conditions RpoS activity appears to protect *E. coli* DNA from reactive oxygen species, DNA repair genes in the LexA regulon are induced, and, the nucleoid-associated protein, H-NS increases the activity of RpoS (Gérard, *et al.*, 1999). When *E. coli* becomes starved for glucose, their growth may be temporarily halted during the diauxic lag phase, during which lactose permease and β galactosidase are induded, thus permitting lactose metabolism (Fischer *et al.*, 1998), In this situation *E. coli* respond by inhibiting σ^S proteolysis, and the level of σ^S rapidly increases. If lactose or another carbon source is available, the diauxic lag phase is followed by a reduction in σ^S synthesis (Fischer *et al.*, 1998).

Dodd and colleagues (1997) noted that the time in which *S. typhimurium* responded to low-temperature challenge was much shorter than the time needed for induction of RpoS, and suggested that the microbial stress response consists of two components, one that occurs very shortly after the stress is imposed, and another system that is RpoS-dependent and induced later. Furthermore, they suggested that during the initial response phase, growth of bacteria stressed by harmful conditions may be inhibited, but environmental stresses do not affect metabolic rate. They proposed this state of imbalance between anabolism and catabolism leads to a relatively high level of free-radical production, which they identified as the 'self-destruction' mechanism that causes cellular death.

Reeve *et al.* (1984) found that the ability of *E. coli* K12 to synthesise protein during the early stages of glucose starvation was an important factor in its ability to withstand further stress. In their experiments they found that the earlier protein synthesis was inhibited, the greater was the reduction in culture half-life. After 9 hours the ability to survive was not changed by the time at which protein synthesis was blocked.

1.5.7 Aggregation Stress Response

Aggregation may also allow consortia of cells to resist the effects of environmental stressors (Gilbert *et al.*, 1993), and indeed may be a pre-adaptation of pathogenic bacteria, which allows them to colonise host tissues (Calleja, 1984).

Peritrichous fimbriae, thin appendages distributed over the surface of bacteria of species such as *Escherichia*, and *Salmonella*, allow cells to pack densely into layers at air-water interfaces. These layers confer an advantage to aerobic or facultative anaerobic bacteria in an aquatic environment, as there is greater access to oxygen at the air-liquid interface (Calleja, 1984).

Another form of defence against inhospitable conditions in surface water is the formation of biofilms. In oligotrophic aquatic environments, mixed or mono-culture bacterial populations attach to surfaces where organic nutrients are more concentrated. Biofilms rarely form under very low nutrient conditions (Atlas, 1997). Within a biofilm in water, bacteria may be protected from grazing protozoa, antibiotics or preservatives in the water (Gilbert *et al.*, 1993). Once a biofilm is established, pathogens can cause bacterial contamination of water supplies, as daughter cells are released into a water distribution system (Olson, 1993; Gilbert *et al.*, 1993).

1.5.8 Pathogen Water Survival Study Methods

Survival study methods vary in experimental simulation conditions (*in-situ* versus *in-vitro* studies), inoculum level and pre-treatment, and enumeration methods. Bacterial survival studies that use diffusion chambers *in- situ* allow bacteria within the chamber access to nutrients at the surface of the chamber membrane. Rhodes and Kator (1988) suggested that bacteria experience more rapid decline during *in-vitro* studies than during *in-situ* studies, because continual nutrient input that occurs *in-situ* typically does not occur *in-vitro*. While the risk of widespread contamination of surface water at an *in-situ* study location may be controlled, even minimal risk of environmental contamination might be unacceptable. Also, more time and labour is involved in obtaining samples from an *in-situ* experiment, than from an *in-vitro* study, because travel to and from the site is required. Thus, *in-vitro* studies are more common.

Initial inoculum levels (C_0) of water-survival studies have ranged from 10^2 (Pokorny, 1988) to $10^{9.3}$ (Muela, *et al.*, 1999). Of 13 studies of pathogen survival in water the median C_0 was $10^{6.2}$. Some workers believe a critical level, 10^6 to 10^7 bacteria/ml, exists, below which enteric bacteria achieve a predator-prey-equilibrium with other micro-organisms; i.e., both predator and prey co-exist without a substantial decline in the level of enteric bacteria (Rhodes and Kator, 1988). A decline below this level is thought to be due to competition for nutrients (Rhodes and Kator, 1988). Washing of cells prior to inoculation into water for survival studies avoids transfer of nutrients from culture media into water. Warburton *et al.*(1994) noted that unwashed cells reacted differently than washed cells. They surmised that the absence of nutrients associated with the washed cells caused a reduction in metabolic rate that induced dormancy, and that this allowed them to survive longer than the unwashed cells.

Plate counts on selective media are simple to perform and are frequently used in studies of survival. Selective media have been used to enumerate specific pathogens in water (Warburton, *et al.*, 1998; Chao *et al.*, 1988; Rhodes and Kator, 1988; Roszak *et al.*, 1983). Wang and Doyle (1998) and Flint (1987) used nalidixic acid-resistant *E. coli* O157:H7 and plated on media with nalidixic acid to avoid enumerating autochthonous bacteria.

Roszak and Colwell (1987) define survival as 'maintenance of viability under adverse conditions'. Some suggest the 'viable but non-culturable' (VBNC) state serves bacteria as a strategy for survival; cellular energy is conserved while environmental conditions are not conducive for growth until favourable conditions return (McKay, 1992). Studies, such as that by Pommepuy *et al.* (1996), which demonstrated that environmentally stressed 'viable but non-culturable' (VBNC) *E. coli* are able to retain their ability to produce enterotoxins reinforce the view that VBNC pathogens may be a health risk (McKay, 1992). Bloomfield, *et al.* (1998) proposed that the resuscitative methods used to enumerate sub-lethally stressed VBNC bacteria cause cell death. They suggested that the proportion of bacteria that survive environmental stress have switched from respiration to substrate-level phosphorylation, with a low rate of superoxide production, and have adapted to stress by inducing RpoS-mediated enzyme production. Immediately following transfer of stress-adapted bacteria to nutrient-rich conditions an instantaneous metabolic imbalance occurs as cell division is decoupled from metabolism. The imbalance leads to overproduction of free radicals and superoxide, and cell death (Bloomfield, *et al.*, 1998).

There are conflicting opinions on the significance and appropriateness of culture techniques used in microbial water survival studies that are concerned with enumerating sub-lethally stressed populations. Roszak and Colwell (1987) list 47 methods to differentiate live from dead cells. Some of the more common methods are outlined below.

Direct viable counts (DVC) enumerate cells with the potential for activity by counting the microscopically elongated bacteria after culture, commonly, in yeast extract with nalidixic acid (Jansson and Prosser, 1997). Nalidixic acid inhibits DNA gyrase, thereby restricting DNA replication, thus it allows an increase in cell volume, while preventing cell division. However, the capability of the cells to replicate is not considered in this method, consequently, DVC may overestimate the potential for growth in culture.

Acridine orange direct counts (AODC) are used to determine total counts without culture. In theory, the AODC method should be able to distinguish between live and dead cells as the acridine orange dye stains nucleic acids differentially, fluorescing orange when cells are rapidly growing and RNA is plentiful, and fluorescing green when only DNA can be seen within the nucleoid of inactive bacteria (Hobbie *et al.*, 1977). Many variations in experimental technique such as pH of dye, incubation time, and concentration of acridine orange, may affect the fluorescent colour. For these reasons, AODC is not considered a highly accurate means of distinguishing live from dead cells (Roszak and Colwell, 1987). Wang and Doyle (1998), Santo Domingo *et al.*(2000), and

Byrd *et al.*, (1991) found little change in AODC of enteric bacteria in water over 12, 45, and 95 weeks, respectively, but observed a population decline using plate counts and DVC.

Immunofluorescence techniques, such as fluorescent antibody (FA) counts are helpful when counts of specific baterial species in a mixed culture are required, but FA methods are unable to differentiate live from dead cells and fluorescing cells may be masked by particulates in water samples (Jansson and Prosser, 1997; Roszak and Colwell, 1987). Roszak *et al.*, (1983) found AODC were 40 and 20% higher than FA counts and DVC, respectively.

Concurrent use of direct counting and culture counting methods in work by Jimenez *et al.*, (1989), Wang and Doyle (1998), Santo Domingo *et al.* (2000), Byrd *et al.*, (1991), Roszak *et al.* (1983) suggest the slope of survival curves is less with direct count methods. The duration of survival of enteric bacteria as measured by DVC has been reported to be shorter than that measured by AODC (Byrd *et al.*, 1991, Roszak *et al.*, 1984). Differences in direct and culture counts have been attributed to the presence of non-culturable or dormant cells (Santo Domingo *et al.*, 2000; Roszak *et al.*, 1983).

Graphical representation of survival of bacteria has been examined in the past (Buchanan *et al.*, 1993; Whiting, 1993; Xiong *et al.*, 1999; Postgate, 1976; Flint, 1987; McCambridge and McMeekin, 1980). Curves generated from concentration values plotted over time for the duration of an experiment exhibit decline phases, but may or may not have other component phases, and frequently vary in shape. These curves may be linear, curved with a shoulder, curved with a tail, or with shoulder and tail (sigmoidal) (Xiong *et al.* 1999).

The initial lag time during which there is little decrease in bacterial concentration with time is termed a shoulder phase. It is thought to be unrelated to the response to environmental conditions (Whiting and Buchanan, 1997). Some have suggested it represents the degree of clumping of cells. Bacterial aggregates are enumerated as one colony forming unit. As cells on the exterior of the aggregate become inactive, the cells within the aggregate survive, thereby extending the duration of survival of the aggregate (Adams and Moss, 1995). Kurath and Morita (1983) reported an increase in respiration of glutamic acid that coincided with the shoulder phase in plots of plate counts versus time. Respiration of glutamic acid decreased sharply after the 5^{th} day of the experiment as did plate counts. But the authors did not suggest a direct association between the two events. In many studies workers have not observed shoulder phases consistently (Postgate, 1976; Flint, 1987; Whiting *et al.*, 1996; Buchanan *et al.*, 1993). There is as yet not an accepted method of determining the transition between phases, but the intersection of the best-fit line through the points of the shoulder period with that of the decline phase has been used to estimate the time at which the bacteria leave the shoulder phase and begin the decline (Xiong *et al.*, 1999).

All bacterial survival curves have some form of decline phase. During this phase, bacteria that have a greater resistance to stress are better able to survive, but eventually they too are unable to persist, and the decrease in this population occurs during the slow count decline of the tail phase. Postgate (1976) suggested that the majority of loss of population seen during the decline phase was a result of the lack of a specific nutrient. If the decline phase is relatively linear, it may be described by first-order kinetics (Hohls *et al.*, 1995; Xiong *et al.*, 1999).

The second lag time that is shown in some survival curves is referred to as the tail phase. Cerf (1977) described biphasic curves with a tail phase as an indication of the presence of two levels of resistance in the bacterial population. Bacteria characterised by Kurath and Morita (1983) as those that had shifted their metabolic activities in response to starvation after 36 days, were able to persist in some samples for up to 1 year.

The overall magnitude of cell loss and duration of survival in non-filtered water varies among studies. McCambridge and McMeekin (1980) observed a decrease of 10^4 *E. coli* counts / ml, with native bacteria, and 10^7 *E. coli* counts / ml, with protozoa and bacteria, in 10 days in estuarine water. Flint (1987) observed a decrease of $10^{6.3}$ *E. coli* viable counts/ml in 2 days in sewage-contaminated water at 37°C, and the same decrease in 10 days at 4°C. Rhodes and Kator (1988) recorded a decrease in *E. coli* counts of approximately 10^3 cells/ml in 11 days in February, and a decrease of 10^2 cells/ml in 15 days in July. In some studies of non-thermal survival models decimal reduction time, D (time/loss of one order of magnitude) is the unit used (Buchanan *et al.* 1993; Whiting, 1993; Xiong *et al.*, 1999).

Mechanistic and empirical models have been developed to predict the survival of bacteria in closed systems. Xiong *et al.* (1999) developed a mechanistic model to predict the time required for a given log cycle reduction in *Staphylococcus aureus* in BHI broth. Their model was a combination of three models. The first order kinetic model (Equation 1-1) describes a survival curve that is linear; the Buchanan model (Equation 1-2) has a linear decline with a shoulder, and the Cerf model (Equation 1-3) has a linear decline with a tail:

$$N(t) = N_o e^{-kt}$$ Equation 0-1

$$\log N(t) = \begin{cases} \log N_o & (t \le t_{lag}) \\ \log N_o - \dfrac{t - t_{lag}}{D} & (t > t_{lag}) \end{cases}$$ Equation 0-2

$$\frac{N(t)}{N_o} = fe^{-k_1 t} + (1-f)e^{-k_2 t}$$ Equation 0-3

where N = concentration of whole population

N_0 = initial concentration

k = death rate constants

D = 2.303k, decimal reduction time

k_1 = death rate of less resistant sub-population (decline phase)

k_2 = death rate of more resistant sub-population (tail phase)

f = initial proportion in the less resistant sub-population

t_{lag}= lag time

A modified Gompertz equation (Equation 1-4) was developed to model sigmoidal survival curves of *Listeria monocytogenes* in sausage, such that the surviving fraction of bacteria was estimated as an exponential function of temperature, pH, and NaCl (Linton, *et al.*, 1995):

$$LSF = Ce^{-e^{(A+Bt)}} - Ce^{-e^{A}}$$ Equation 0-4

where LSF = log surviving fraction of bacteria

A = f (temperature,pH,[NaCl]), effect on shoulder phase

B = f (temperature, pH, [NaCl]), effect on death rate

C = f (temperature, pH, [NaCl]), related to total change in cell concentration

Such models of inactivation in foods are useful in permitting the estimation of the effect of food processing and handling on bacterial survival. However, these closed models may not accurately predict the survival in aquatic systems as they are typically prone to fluxes of organic matter, micro-organisms, and other waterborne biota (Stevenson, 1978).

2 Prevalence of *Escherichia coli* O157:H7 and *Salmonella* in Surface Waters of Southern Alberta

2.1 Introduction

Public concern about microbial water quality and the need to understand the occurrence of bacterial pathogens in surface water systems is evident, particularly following waterborne outbreaks of enteric disease, such as in Walkerton, Ontario (Kondro, 2000). While the use of untreated surface water for drinking is low in the general population, contamination of public water supplies by surface water containing *E. coli* O157:H7 and other pathogens has been reported (Jones and Roworth, 1996; Barwick *et al*, 2000). Disease outbreaks caused by *E. coli* O157:H7 in recreational water have also occurred in the past (Keene *et al*, 1994; Kramer *et al.*, 1996; Warner *et al.*, 1996).

Indicator organisms such as faecal coliforms and *E. coli* bacteria are often used to suggest faecal contamination in materials, such as food and water. The strength of these estimators to predict the presence of pathogens known to cause disease in humans has differed among studies, and these differences may be due to contamination level, laboratory methods of enumerating indicator organisms and the ability to detect pathogens (Polo *et al.*, 1998; Arvanitidou *et al.*, 1997; Townsend, 1992; Luppi, *et al.* 1988; Efstratiou *et al.*, 1998). The guideline limits, or maximum allowable concentration for water use in Alberta during 1999/2000 were: 400 *E. coli* or faecal coliforms /100ml in recreational water (Natural Resources Service, Water Management Division, Alberta Environment, 1999); zero *E. coli* 100ml in drinking water, although if there are no *E. coli* in a sample, up to 10 total coliforms are considered acceptable

(Federal-Provincial Subcommittee on Drinking Water, 1999); 1000 total coliforms /100ml and 100 faecal coliforms /100ml in irrigation water (Canadian Council of Ministers of the Environment, 1999).

This study was designed to gain information needed to understand the health risks associated with *E. coli* O157:H7 and *Salmonella* spp in surface water in southern Alberta by estimating the prevalence of these pathogens in surface waters within the Oldman River basin in southern Alberta. The hypotheses were that these pathogens were present in surface water and that their prevalence varied with time and location. Temporal trends in the frequency of detection of these pathogens were observed. A comparison between the rate of detection in 1999 and 2000 for sites tested in both years was performed to detect annual variability. As well, variation in prevalence among sampling sites was observed. Three *Salmonella* -selective media were used in 2000 and a comparison of their effectiveness in the detection of *Salmonella* was made. The relationship between indicator organism concentration and the presence of *E. coli* O157:H7 and *Salmonella* spp. in the water samples tested in this study was also examined. As well, comparisons were made between the rating of water samples in terms of the microbial water quality guidelines used in Alberta (Natural Resources Service, Water Management Division, Alberta Environment, 1999; Federal-Provincial Subcommittee on Drinking Water, 1999; Canadian Council of Ministers of the Environment, 1999) and the presence of *E. coli* O157:H7 and *Salmonella* spp.

2.2 Methods and Materials

2.2.1 Study Sites

The Oldman River watershed in southern Alberta drains approximately 26,700 km^2 (Natural Resources Canada, 2001). The headwaters of the Oldman River are along the crest of the Rocky Mountain range. The river flows for 362 km until reaching the confluence with the South Saskatchewan River. The watershed includes foothills east of the Rocky Mountains and, further east, a generally flat topography with riverine coulees. The land east of the foothills is largely agricultural, including dry land and irrigated crop farming.

Of the 60261 km^2 of farmland in southern Alberta, 94% (64107 km^2) is irrigated (Su, 1996). Primary crops within irrigated regions include barley, canola, and alfalfa (Alberta Agriculture, Food and Rural Development, 2000). In Alberta many high animal-density beef cattle operations are situated in regions serviced by irrigation distribution systems. Primary feeds used in these operations are irrigated barley silage and grain barley (Alberta Agriculture, 1991). Dryland agriculture includes crops such as triticale, safflower, lentils, as well as cattle grazing operations.

The Lethbridge Northern Irrigation District (LNID) is one of 13 irrigation districts in Alberta. Its distribution system conveys water used to irrigate 534 km^2 of agricultural land within the Oldman River basin. The water is also used for livestock watering, recreation and residential purposes (Lethbridge Northern Irrigation District, 2000). Mean monthly air temperature between April and October in Lethbridge ranges from 5.5 to 18.0°C, and mean monthly precipitation from 22.0 to 74.0mm (Agriculture and Agri-food Canada Research Branch, 2000). From May 3 to September 7, 1999, water temperature within the LNID distribution system ranged from 3^0C to 23^0C (LNID, personal communication).

In 1999, surface water samples used in this study came from 62 sites within the Oldman River watershed, including 14 within the LNID (Appendices 1-3). Sampling sites within the LNID water were adjacent to agricultural, recreational, or residential land. Some sites were downstream of urban centres (Lethbridge, Fort Macleod, Picture Butte, Coaldale); the samples from those sites included urban storm water runoff, industry and municipal sewage treatment effluent, and groundwater recharge from those cities. As well, samples were also collected from irrigation water at intakes of rural municipalities (10 sites). Additional sites were tested in two series of synoptic flow samples.

In 2000 additional sampling sites included 9 within the storm drain system of Lethbridge, Alberta (population 68,712), 6 different municipal sewage treatment plants, 3 sites around a ski hill, and 16 sites along the Little Bow River and its tributaries.

2.2.2 Sample Collection

Over the 2-year duration of the study, 97 separate sites were sampled regularly (weekly, bi-weekly, tri-weekly, or monthly). Regularly sampled sites are shown in Appendices 1-3. In 1999, 62 sites were sampled regularly; in 2000, 58 were sampled regularly. In addition, samples were obtained occasionally (1 or 2 samples from each) from other sites, including sewage treatment facilities. Twenty-three sites were sampled regularly in both years.

Staff of Water Management Division, Natural Resources Service of Alberta Environment; Irrigation Branch of Alberta Agriculture, Food and Rural Development;

Chinook Health Region; LNID; and City of Lethbridge collected the water samples. Samples were taken either upstream from foothold, or with a plastic container on a pole, from water at approximately 30 cm below the surface. Microbial analysis was conducted at three laboratories. The Health Canada laboratory, Lethbridge, tested water samples for the presence of *E. coli* O157:H7 and *Salmonella*. Some water samples were tested for indicator organism concentration at University of Lethbridge Water Resources Institute and the balance at the Provincial Laboratory of Public Health for Southern Alberta. Water samples tested at the Health Canada laboratory and the University of Lethbridge were collected in, or transferred to, sterile plastic bottles, and transported on ice packs and laboratory analysis commenced within 24 hours of collection. Samples tested at the Provincial Laboratory of Public Health were collected in, or transferred to, sterile plastic bottles with sodium thiosulfate (to inactivate chlorine), and were transported to the lab and tested before the end of the next day.

From June 1 to December 8, 1999, 586 and 530 water samples from 70 sites were tested for the presence of *E. coli* O157:H7 and *Salmonella*, respectively. From March 3 to October 14, 2000, 967 samples from 58 sites were tested for the presence of these pathogens.

2.2.3 Detection of *E. coli* O157:H7

Water samples were dispensed in 100 ml volumes and 10 ml of 10X buffered peptone water (BPW) was added, and incubated at 37°C for approximately 6 h. At this point, 1 ml was withdrawn, added to 9-ml trypticase soy broth with 2% novobiocin (Difco, Detroit), and held at 42°C for 6 h ± 2 h. Immuno-magnetic separation using magnetic beads (Dynal, Oslo) coated with O157 antibody was carried out as described by Chapman, *et al.* (1994). Bacteria adhering to the beads were streaked onto sorbitol MacConkey agar containing cefixime (0.05 mg/l) and tellurite (2.5 mg/l)(Sanderson *et al.* 1995). Following 24-h incubation at 42 ^{o}C, translucent colonies were tested by slide agglutination using O157-specific antibody (Difco, Detroit). Agglutinating bacterial colonies were inoculated into brain heart infusion broth (BHI) (Difco, Detroit). The bacterial growth in BHI was harvested by centrifugation and DNA was extracted from the bacterial growth as described by Ausubel *et al.* (1995). The DNA was tested using a polymerase chain reaction (PCR) using the oligonucleotide primers that detect vt_1, vt_2, *fliC*, and $eae\mathrm{A}_{O157}$ genes, the combination that suggests the presence of *E. coli* O157:H7 (Gannon *et al.* 1997).

2.2.4 Detection of *Salmonella*

Water samples cultured in BPW, a pre-enrichment media, described above, were incubated for another 18 h at 37^{o}C. In the first year of the study, a 1 ml aliquot of was taken from the BPW culture, inoculated into 9 ml of Rappaport Vassiliadis broth (RV) for selective enrichment, and the culture was incubated at 42^{o}C for 24 h. In 2000 the ratio of BPW culture to RV broth was 1/100. In both years a 20 µL aliquot of the culture was dropped onto the surface of modified semi-solid Rappaport Vassiliadis Agar (MSRV) (DeSmedt *et al.*, 1986; DeSmedt and Bolderdijk, 1987). If halos greater than 20 mm in diameter formed in the media following another 24h incubation, a loopful of media at the edge of the halo was cultured on MacConkey agar, and colonies were tested by a slide agglutination test with *Salmonella* O antiserum Poly A-1 and Vi (Difco, Detroit).

To supplement the *Salmonella* testing in the second year, testing included plating samples, following selective enrichment in tetrathionate brilliant green broth (TBG), on bismuth sulphite agar (BS), and brilliant green sulpha agar (BGS) (Becton Dickinson, Cockeysville) as described by D'Aoust and Purvis (1998). Suspected *Salmonella* colonies on BS are shiny, dark greenish-brown with darkening of the surrounding media without clearing, and on BGS are pink surrounded by pink media.

In the 2000 season, putative *Salmonella* colonies were inoculated into (Christensen's) Urea agar, triple sugar iron agar (TSI) and lysine iron agar (LIA) slants (D'Aoust and Purvis, 1998), and SIM medium (Carter, 1979). Those colonies exhibiting biochemical responses typical of *Salmonella* were further tested using a multiplex PCR specific for the detection of the *Salmonella invA* gene (Chiu and Ou, 1996).

Putative *Salmonella* cultures were sent to the Health Canada *Salmonella* Reference Laboratory in Guelph, Ontario for biochemical testing and serotyping.

2.2.5 Indicator Organism Enumeration

Water samples were grouped by the site from which they were obtained and the method by which they were enumerated.

2.2.5.1 Sample Set 1

The Provincial Laboratory of Public Health for southern Alberta received water samples from regularly sampled Sites 1 through 76, (Appendices 1 and 3) and sewage treatment facility samples the day after sampling. Tests were performed on the day after sampling. Enumeration of indicator organisms was performed using a membrane filtration technique (0.45 μm pore)(Provincial Laboratory of Public Health, Calgary, personal communication). Filters were placed on faecal coliform agar modified (mFC) with rosolic acid, to determine faecal coliform counts and on lactose glucoronide agar to determine *E. coli*. In both cases incubation was at 44.5°C for 24 h ± 2 h. Blue, and green or blue colonies were counted using stereoscopic microscopy as faecal coliform and *E. coli*, respectively.

2.2.5.2 Sample Set 2

The University of Lethbridge Water Resources Institute enumerated indicator organisms in water collected from Sites 77 though 97 (Appendix 3). Samples were tested within the same day of sample collection. Samples volumes of 1 ml to 150 ml (adjusted to obtain counts) were filtered (0.45 µm pore) and placed on mFC agar (Difco, Detroit). In 1999 cultures were incubated overnight at 37°C to obtain total coliform counts and at 42°C or 45°C to obtain faecal coliform counts. Blue colonies were counted as faecal coliforms and pink colonies as total coliforms. In 2000 plates were incubated for 24 hours at 42°C. Concentration was taken as the mean count of three plates per sample.

2.2.5.3 Statistical Methods

The apparent prevalence of *E. coli* O157:H7 and *Salmonella* in the water samples was estimated as the number of samples from which pathogens were detected over the number of samples tested. Prevalence in sampling sites tested in both years was compared using a paired sample *t*-test. Sensitivity of *Salmonella* detection methods was calculated based on *Salmonella* PCR results using the standard calculation:

$$SENS = \frac{TP}{TP + FN} \qquad \text{Equation 0-1}$$

where: TP = samples that were *Salmonella* PCR-positive and culture positive on media

FN = samples that were *Salmonella* PCR-positive and culture negative on media

Indicator organism counts were tested statistically as two separate pools (Sample Sets 1 and 2) to minimise any effects of different laboratory technique and sample transport time. Since the *Standard Methods for the Examination of Water and Wastewater* (American Public Health Association, 1985) recommends incubation at 44.5 ± 0.2^{0}C to obtain faecal coliform counts in water, 45^{0}C was assumed to have little selective effect against faecal coliforms, hence faecal coliform concentrations in Sample Set 2 determined with 45^{0}C and 42^{0}C during 1999 and 2000 were pooled for analysis.

The Mann-Whitney U-test was used to compare mean ranks of indicator organism counts in samples with or without pathogens (Polo *et al.*, 1998). Univariate logistic regression analysis was performed to describe any relationship between probability of pathogen presence or absence based on concentration of indicator organisms (Efstratiou *et al.*, 1998; Hosmer and Lemeshow, 1989).

Significance of association between the violation/non-violation status of samples with respect to water use guidelines and presence/absence of pathogens was tested with Fisher's Exact-test of 2X2 contingency tables. When significance of association was demonstrated, association was estimated using an adaptation of the relative risk (RR), or cumulative incidence ratio equation (Gertsman, 1998).

$$RR = \frac{TP}{TP+FP} \Big/ \frac{FN}{FN+TN}$$ Equation 0-2

where: FP = samples that were *Salmonella* PCR-negative and culture positive on media

TN = samples that were *Salmonella* PCR-negative and culture negative on media

The predictive value positive (PVP) and predictive value negative (PVN) (Gertsman, 1998) were estimated as:

$$PVP = \frac{TP}{TP+FP}$$ Equation 0-3

and

$$PVN = \frac{TN}{TN+FN}$$ Equation 0-4

PVP and PVN were calculated to assess the effectiveness of water use guidelines as a means of predicting the presence of pathogens and of *Salmonella*- selective plating media as a means of detecting isolates confirmed as *Salmonella.*

2.3 Results

2.3.1 Prevalence of Pathogens

In 1999 and 2000, *E. coli* O157:H7 was isolated from 13 of 1520 water samples (0.86%); *Salmonella* were detected in 88 of 1456 samples (6.04%). *Salmonella* and *E. coli* O157:H7 were both detected in samples from the Fort Macleod sewage treatment plant on 8 September 2000, and West Side outflow on 31 July 2000. The latter sample was from one of three sites, each on separate water flow paths, from which *E. coli* O157:H7 was detected on the same day. It was also detected on two sampling days from the Oldman River downstream of the Battersea drain (Table 2-1), which receives agricultural irrigation drainage water.

Salmonella serovars isolated were: Agona, California, Cubana, Enteriditis, Give Var.15+, Hadar, Heidelberg, Indiana, Mbandaka, Newport, Rubislaw, Schwarzengrund Senftenberg, Thompson, Typhimurium, Typhimurium Var. Copenhagen, I:4,5,12:i:-, I:4,12:i:-, I:6,8:eh:-, I:11:r:-, I:Rough-O:r:-, I:Rough-O:r:enx, IIIa:41:z4,z23:, IIIa:42:g,z51:-, IIIb:16:z10:enxz15, IIIb:60:r:-, IIIb:60:r:enxz15. Typically each sample contained only one *Salmonella* serovar, however, two different serovars were isolated from 6 samples, hence, in total 94 *Salmonella* were isolated (Table 2-2). A large proportion of the isolates (36.8%) were *S. rubislaw*.

Salmonella was detected in more than one sample from 19 different sites, i.e, from samples collected from the same site but on different sampling days (Table 2-3). The sites of highest *Salmonella* prevalence were the Fort Macleod sewage treatment plant sample (prevalence =100%, n =1), followed by Storm Drain N2 (prevalence =80%, n = 5) (Table 2-3). Sites where *Salmonella* prevalence was 33.3% and 25% included 2 sites that receive irrigation drainage from areas of livestock agriculture, LB6-9A and S1-DC (Alberta Agriculture, Food and Rural Development, personal communication), and one that received drainage from a goose pond, K-1 spill outflow (LNID, personal communication).

In 2000, the prevalence of *E.coli* O157:H7 peaked in the middle to end of July (Figure 2-1). The mid-summer peak in *Salmonella* prevalence was common to both years (Figures 2-2 and 2-3). The maximum weekly *Salmonella* prevalence during the study was during the week of July 24, 2000 when 14 of 42 samples (33.3%) contained the pathogen (Figure 2-3; serovars listed in Table 2-4).

The overall proportion of water samples that exhibited growth typical of *Salmonella* on MSRV was 4.06% (8 of 197) in 1999 and 6.14% (18 of 293) in 2000 (data not shown). Although this is an increase from one year to the next, there was no significant difference in rate of *Salmonella* detected using MSRV in water from sites tested in both years between the two years (Table 2-5)(paired sample test t= 1.116; 2-tailed P =0.275; df=24). There was, however, a significant increase in the rate of *E. coli* O157:H7 detection in water from sites sampled in both years (Table 2-6)(paired samples test, t = 1.809, 2-tailed P=0.0830; df=24). Prevalence of *E. coli* O157:H7 and *Salmonella* was greater in sewage treatment plant and storm drain samples than in samples from other types of sites (Table 2-7).

Of the 74 samples confirmed as *Salmonella* in 2000, 66, 52, and 58 samples exhibited growth typical of *Salmonella* on MSRV, BGS, and BS, respectively. The calculated sensitivity of MSRV, then, was 89.2%, while the sensitivities of BGS and BS were less, 70.3% and 78.4%, respectively (Table2-8). The number of samples from which there was growth typical of *Salmonella* on MSRV, BGS, and BS were 74, 407, and 348, respectively. Thus, the predictive value positive for MSRV, BGS, and BS was 0.89, 0.13, and 0.17, respectively. This suggests that the likelihood that a sample that was presumed to be *Salmonella* based on the growth on the selective media and would later be confirmed to contain *Salmonella* was considerably greater if the media was MSRV, than if it was BGS or BS.

Table 2-1 Date and location of water samples from which *E. coli* O157:H7 was isolated. Refer to Appendices 1, 2, and 3 for locations.

Date	Sample Site
12 July 1999	Six Mile Coulee near Carlson Livestock
7 June 2000	S1-DC
21 June 2000	LB5M
4 July 2000	Oldman River downstream of Battersea drain
17 July 2000	Oldman River downstream of Battersea drain
31 July 2000	E-6 outflow
31 July 2000	Park Lake inflow
31 July 2000	West Side outflow
16 Aug. 2000	LB3M
30 Aug. 2000	S2-DC
8 Sept. 2000	Fort Macleod Sewage Treatment Plant
18 Sept. 2000	A-1 at MacIntyre section
25 Sept. 2000	West Side inflow

Table 2-2 *Salmonella* isolates obtained during 1999 and 2000.

Serovar	Number of isolates	%
Agona	2	2.1
California	2	2.1
Cubana	1	1.1
Enteriditis	1	1.1
Give Var.15+	6	6.3
Hadar	2	2.1
Heidelberg	2	2.1
Indiana	1	1.1
Mbandaka	5	5.3
Newport	2	2.1
Rubislaw	35	36.8
Schwarzengrund	1	1.1
Senftenberg	1	1.1
Thompson	1	1.1
Typhimurium	2	2.1
Typhimurium Var. Copenhagen	4	4.2
I:4,5,12:i:-	1	1.1
I:4,12:i:-	4	4.2
I:6,8:eh:-	1	1.1
I:11:r:-	2	2.1
I:Rough-O:r:-	4	4.2

Table 2-2 continued

I:Rough-O:r:enx	4	4.2
IIIa:41:z4,z23:-	1	1.1
IIIa:42:g,z51:-	1	1.1
IIIb:16:z10:enxz15	3	3.2
IIIb:60:r:-	1	1.1
IIIb:60:r:enxz15	4	4.2
Total	94	100

Table 2-3 *Salmonella* serovars listed by sample site.

Site name	Prevalence (*n*)	Serovars
B1M	5.0 (20)	Rubislaw
B4-4	10.0 (20)	I:rough-O:r:- / Give var. 15+
B4-5	15.0 (20)	I:rough-O:r:enx / Give var. 15+ / Rubislaw (2)
B4M	10.0 (20)	Rubislaw (2)
LB4-2	20.0 (20)	Mbandaka / I:rough-O:r:enx Give var. 15+ / Rubislaw
LB4M	10.0 (20)	I:rough-O:r:- / Rubislaw
LB5-10	10.0 (20)	I:4,12:i:- / I:rough-O:r:enx / Rubislaw
LB5M	20.0 (20)	I:4,12:i:- / Rubislaw (3)
LB6-9A	25.0 (20)	Newport (2) / I:6,8:eh:- / I:rough-O:r:enx IIIb:16:z10:enxz15 / Rubislaw (2)
S1-DC	25.0 (16)	Give var. 15+ / Rubislaw (2) / Enteriditis
S2-DC	5.3 (19)	Mbandaka
Beaver Creek	7.7 (13)	Give var. 15+
Coalhurst Sewage Treatment Plant	100 (1)	Heidelberg

Table 2-3 continued

Fort Macleod Sewage Treatment Plant	100 (1)	Agona
Little Bow River	7.1 (14)	Rubislaw
Oldman River above Lethbridge	16.7 (12)	Rubislaw (2)
Oldman River north of Taber	13.3 (15)	I:4,12:i:- / I:4, 5,12:i:-
Six Mile Coulee spillway	15.0 (20)	IIIb:60:r:enxz15 / IIIb:60:r:- / Rubislaw
Six Mile Coulee upstream of StormDrain 94-S8	15.4 (13)	Give var. 15+ / IIIb:60:r:enxz15
Six-Mile Coulee / Whoop-up spillway	12.5 (8)	IIIb:60:r:enxz15
St Mary River	10.0 (10)	Rubislaw
Storm Drain S-5/6	20.0 (5)	Hadar
Storm Drain at Churchill Pond	25.0 (4)	Cubana
Storm Drain N-2	80.0 (5)	California / Heidelberg/ Mbandaka Schwarzengrund/ Senftenberg

Table 2-3 continued

Storm Drain S-3	20.0 (5)	Thompson
Storm Drain S-7	20.0 (5)	Rubislaw
Storm Drain W-3	20.0 (5)	California
Storm Drain 94-S8	20.0 (5)	Mbandaka
61-C inflow	9.7 (31)	Typhimurium / I:11:r / Hadar
A-1 @ Hwy 2	13.6 (22)	Rubislaw (2) / IIIb:16:z10:enxz15
A-1 @ MacIntyre	9.1 (22)	IIIb:60:r:enxz15 / Rubislaw
A-1 outflow	13.3 (30)	Mbandaka / Rubislaw (3)
A-1 inflow	3.3 (30)	IIIb:60:r:enxz15
Albion split	50.0 (2)	Typhimurium
Battersea drain	9.1 (22)	Rubislaw (2)
K-1 Spill inflow	16.7 (6)	I:11:r
K-1 Spill outflow	33.3 (6)	Agona, Indiana
Monarch drain	12.1 (33)	I:rough-O:r:- / Rubislaw (2)
		Typhimurium var. copenhagen (2)
Monarch main	3.0 (33)	Rubislaw
Oldman River	13.6 (22)	IIIa:41:z4,z23:- / I:4,12:i:- / IIIa:42.g,z51:-
downstream of Battersea		

Table 2-3 continued

Park Lake south	4.3 (23)	Typhimurium var. copenhagen
Piyami	3.0 (33)	Rubislaw
Szteina drain	12.1 (33)	I:rough-O:r:- / Rubislaw (2) Typhimurium var. copenhagen
West side outflow	4.8 (21)	IIIb:16:z10:enxz15

Parentheses indicates number of times a serovar was detected, when detected more than once.

Table 2-4 *Salmonella* detected by week.

Week starting:	Isolates	*Salmonella* serovars
12-Jul-99	2	Agona / IIIb:60:r:enxz15
26-Jul-99	1	Hadar
09-Aug-99	3	Rubislaw (3)
16-Aug-99	2	Give var. 15+/ Rubislaw
23-Aug-99	2	Indiana / Typhimurium
06-Sep-99	4	Rubislaw (2) / I:11:r:- (2)
22-May-00	2	Mbandaka / Give Var. 15+
29-May-00	2	IIIa:41:z4,z23:- / Mbandaka
05-Jun-00	1	I:4,12:i:-
12-Jun-00	1	I:4,12:i:-
19-Jun-00	1	I:4,12:i:-
26-Jun-00	1	[I:6,8:eh:- / Newport]
03-Jul-00	1	IIIb:60:r:enxz15
10-Jul-00	3	Typhimurium / IIIb:60:r:enxz15 / Mbandaka
17-Jul-00	4	I:rough-O:r:- / I:rough-O:r:enx /Newport /Rubislaw
24-Jul-00	14	Rubislaw (6) / I:rough-O:r:enx (2) / I:rough-O:r:-(2)
		Give Var.15+(2) / Heidelberg
		[Rubislaw / I:rough-O:r:enx]

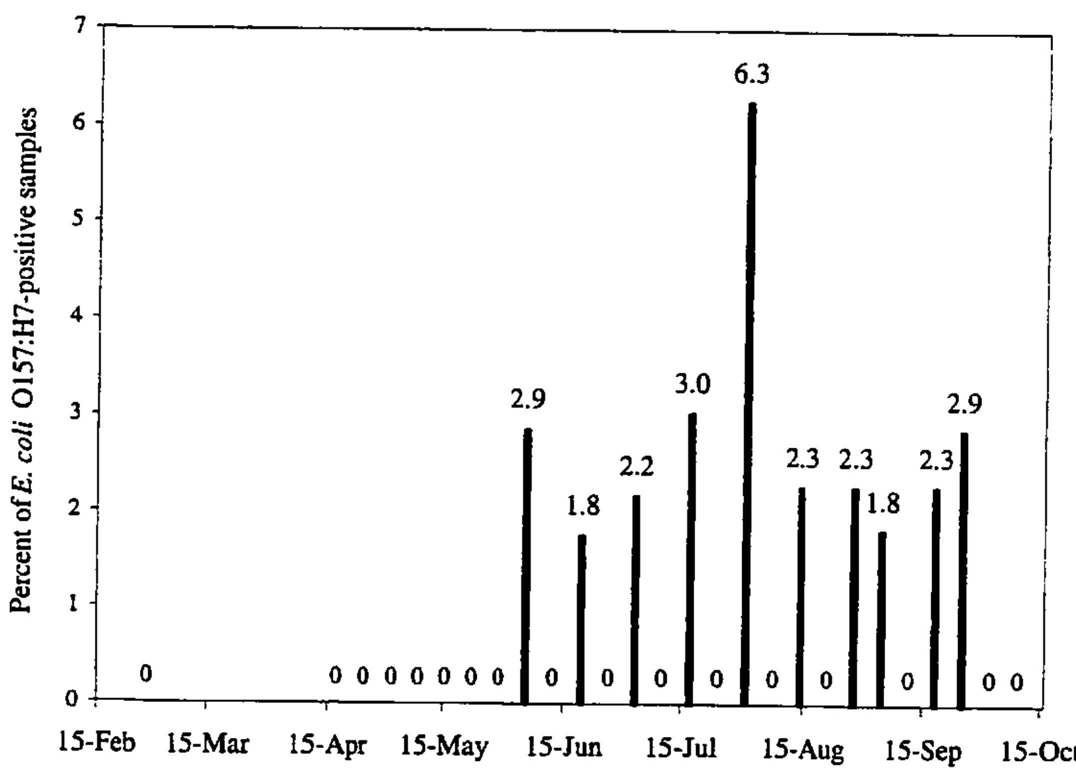
Table 2-4 continued

31-Jul-00	15	Rubislaw (8) / IIIa:42.g,z51:- / IIIb:16:z10:enxz15 Enteriditis / [I:4,12:i:- / I:4, 5,12:i:- [IIIb:60:r:-/ Rubislaw] / [Give Var.15+ / Rubislaw] [IIIb:16:z10:enxz15 / Rubislaw]
07-Aug-00	7	Rubislaw(6) / Typhimurium var. copenhagen
14-Aug-00	3	California / Rubislaw/IIIb:60:r:enxz15
21-Aug-00	3	IIIb:16:z10:enxz15 / Typhimurium var. copenhagen / Give var 15+
28-Aug-00	8	Typhimurium var. copenhagen / Rubislaw(2) Mbandaka Hadar / Thompson / Senftenberg / Schwarzengrund
04-Sep-00	2	Rubislaw / Agona
11-Sep-00	3	Typhimurium var. copenhagen / IIIb:60:r:enxz15 Heidelberg
18-Sep-00	2	Mbandaka / Cubana

Square brackets indicate that two different serovars were found in one sample.

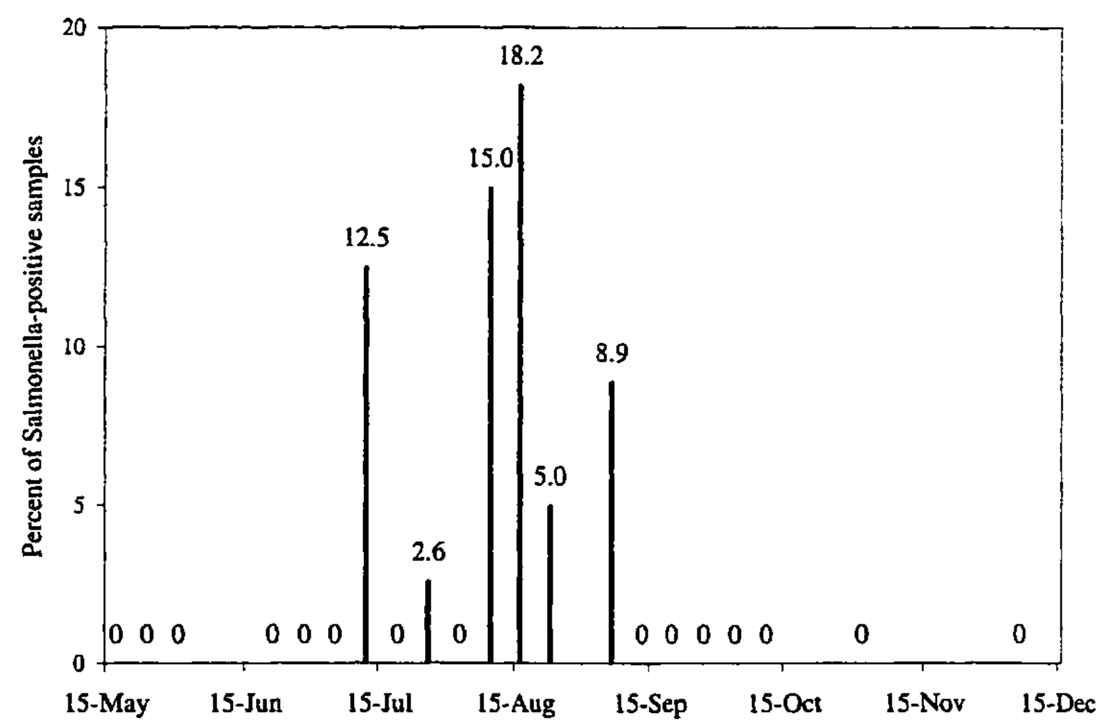
Parentheses indicate number of times a serovar was detected, when detected more than once.

Figure 2-1 *E. coli* O157:H7 prevalence by week in 2000, $n = 967$



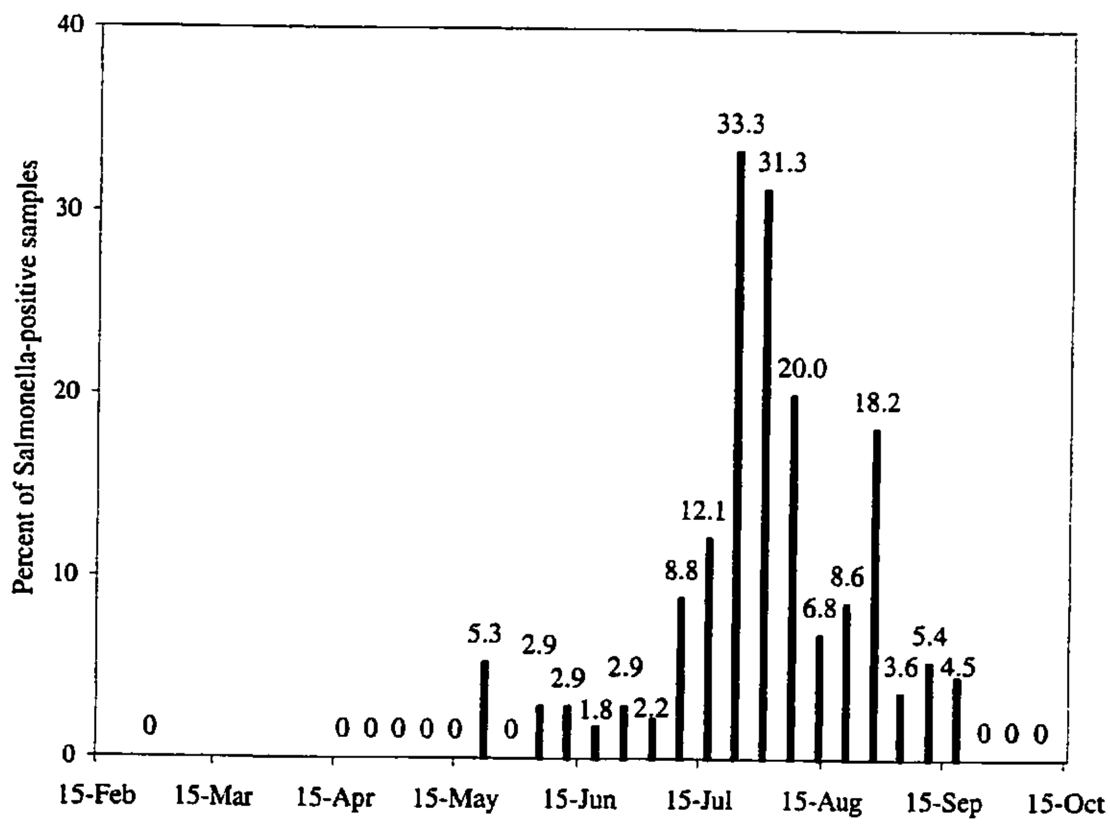
No samples were tested during weeks where no values are shown.

Figure 2-2 *Salmonella* prevalence by week in 1999, n= 491.



No samples were tested during weeks where no values are shown.

Figure 2-3 *Salmonella* prevalence by week in 2000, n=967.



No samples were tested during weeks where no values are shown.

Table 2-5 *Salmonella* prevalence by site and year. Sites listed are those that were sampled in 1999 and 2000.

Sampling site	% Prevalence (n)	
	1999	2000
Battersea Drain	0.0 (7)	0.0 (6)
Beaver Creek	11.1 (9)	0.0 (4)
Castle River	0.0 (7)	0.0 (6)
Expanse Coulee Adjacent To Highway #36 Bridge	0.0 (9)	0.0 (7)
Haney Drain	0.0 (2)	0.0 (7)
Little Bow River Near The Mouth	14.2 (7)	0.0 (7)
Oldman River Above Lethbridge	14.2 (7)	16.7 (6)
Oldman River At Highway # 36 Bridge North Of Taber	0.0 (6)	14.2 (7)
Oldman River Near Brocket	0.0 (6)	0.0 (6)
Oldman River Near Purple Springs	0.0 (6)	0.0 (2)
Piyami Drain	0.0 (9)	0.0 (6)
Six Mile Coulee Spillway	0.0 (15)	40.0 (5)
Six Mile Coulee Upstream Of Storm Drain 94-S8	0.0 (8)	40.0 (5)
Monarch drain	14.2 (7)	18.2 (22)
A-1 inflow	0.0 (8)	4.5 (22)

Table 2-5 continued

A-1 outflow	12.5 (8)	13.6 (22)
Monarch main	0.0 (8)	4.5 (22)
Piyami	11.1 (9)	0.0 (22)
E-6 outflow	0.0 (8)	0.0 (22)
Park Lake inflow	0.0 (9)	0.0 (22)
61-C inflow	22.2 (9)	4.5 (22)
Szteina drain	0.0 (8)	18.2 (22)
West side inflow	0.0 (9)	0.0 (22)

Table 2-6 *E. coli* O157:H7 prevalence by site. Sites listed are those that were sampled in 1999 and 2000.

Sampling site	% Prevalence (*n*)	
	1999	2000
Battersea Drain	0.0 (7)	0.0 (6)
Beaver Creek	0.0 (9)	0.0 (4)
Castle River	0.0 (7)	0.0 (6)
Expanse Coulee Adjacent To Highway #36 Bridge	0.0 (9)	0.0 (7)
Haney Drain	0.0 (2)	0.0 (7)
Little Bow River Near The Mouth	0.0 (7)	0.0 (7)
Oldman River Above Lethbridge	0.0 (7)	0.0 (6)
Oldman River At Highway # 36 Bridge North Of Taber	0.0 (6)	0.0 (7)
Oldman River Near Brocket	0.0 (6)	0.0 (6)
Oldman River Near Purple Springs	0.0 (6)	0.0 (2)
Piyami Drain	0.0 (9)	0.0 (6)
Six Mile Coulee Spillway	0.0 (15)	0.0 (5)
Six Mile Coulee Upstream Of Storm Drain 94-S8	0.0 (8)	0.0 (5)
Monarch drain	0.0 (7)	0.0 (22)
A-1 inflow	0.0 (8)	0.0 (22)

Table 2-6 continued

A-1 outflow	0.0 (8)	0.0 (22)
Monarch main	0.0 (8)	0.0 (22)
Piyami	0.0 (9)	0.0 (22)
E-6 outflow	0.0 (8)	4.5 (22)
Park Lake inflow	0.0 (9)	4.5 (22)
61-C inflow	0.0 (9)	0.0 (22)
Szteina drain	0.0 (8)	0.0 (22)
West side inflow	0.0 (9)	4.5 (22)

Table 2-7 Number of isolates from various types of sampling locations, 2000.

Species	Sewage treatment plant (%) $n = 6$	Storm drain (%) $n = 43$	Watershed [a] (%) $n = 912$
E. coli O157:H7	1 (16.67)	0 (0.00)	11 (1.21)
Salmonella	2 (33.33)	12 (27.91)	60 (6.58)

[a] Watershed samples were from sites that were not at sewage treatment plants or in the storm drain system.

Table 2-8 Effectiveness of *Salmonella* isolation methods in 2000. The number of samples confirmed to be *Salmonella*-positive by serotyping and PCR in 2000 was 74.

Detection method	True positive samples	False positive samples	Sensitivity (%)	Percent value positive
MSRV	66	0	89.2	0.89
BS	52	355	70.3	0.13
BGS	58	290	78.4	0.17

2.3.2 Pathogen Presence and Indicator Organism Concentration

There appeared to be variability in the degree to which pathogen presence could be inferred from indicator organism concentration in water samples. Differences in mean, median, and range of 50% of indicator organism counts of pathogen-positive and pathogen-negative samples in Sample Set 1(counts of samples from Sites 1 through 76, performed at the Provincial Laboratory of Public Health)(Table 2-9, Figures 2-4 and 2-5) suggest that among these samples, the presence of pathogens was generally more likely in water samples with higher faecal coliform and *E. coli* counts. The predictive nature of counts in Sample Set 2 (counts of samples from Sites 77 through 97 performed at the University of Lethbridge) was less than that of Sample Set 1. In Sample Set 2 only the faecal coliform counts of *Salmonella*-positive and negative samples were significantly different ($P = 0.000$) (Table 2-10). Furthermore, the mean and range of 50% of faecal coliform counts of *E. coli* O157:H7-positive samples in Sample Set 2 were lower than that of *E. coli* O157:H7-negative samples (Table 2-10, Figure 2-4).

Pathogens were detected in 3 different samples in Sample Set 2 whose faecal coliform concentration was measured at zero CFU/100ml. These samples include one sample each on July 17, 2000 and September 25, 2000 from which *E. coli* O157:H7 was detected, and one sample on September 4, 2000, in which *Salmonella* was detected.

The probability of the presence of pathogens in water samples was not associated with indicator organism counts. Logistic regression coefficients were close to zero in models of pathogen-indicator organism relationships (data not shown).

There was a lack of significant association between the violation or non-violation of a sample with respect to the guidelines for indicator organism concentration and the presence or absence of *E. coli* O157:H7 (Tables 2-11 and 2-12). There was, however, a significant association between the risk of *Salmonella* presence and violation of water use guidelines for faecal coliforms in recreational and irrigation water (Tables 2-11 and 2-12). Drinking, irrigation, and recreational water microbial guidelines were generally strong in terms of their predictive values negative, indicating that the absence of pathogens could be effectively predicted in samples that were below the guidelines for microbial water quality (Tables 2-11 and 2-12). Conversely, predictive values positive were generally low, thus presence of *Salmonella* and *E. coli* O157:H7 could not be assumed in samples that were above the guidelines.

Table 2-9 Sample Set 1 mean count of indicator organisms in 1999 and 2000. *P* is the asymptotic 2-tailed significance of Mann-Whitney U-statistic, testing for difference in counts between pathogen-positive and pathogen-negative samples.

Indicator	Pathogen	Pathogen-positive (CFU/100ml)	Pathogen-negative (CFU/100ml)	*P* (*n*)
Faecal coliforms	*Salmonella*	5758.98	620.78	0.000 (804)
	E. coli O157:H7	12388.20	880.69	0.003 (805)
E. coli	*Salmonella*	1573.71	267.10	0.000 (804)
	E. coli O157:H7	2260.20	339.49	0.004 (805)

Table 2-10 Sample Set 2 mean count of indicator organisms in 1999 and 2000. *P* is the asymptotic 2-tailed significance of Mann-Whitney U-statistic, testing for difference in counts between pathogen-positive and pathogen-negative samples.

Indicator	Pathogen	Pathogen-positive (CFU/100ml)	Pathogen-negative (CFU/100ml)	*P* (*n*)
Faecal	*Salmonella*	1072.16	295.36	0.000 (518)
coliforms	*E. coli* O157:H7	106.03	349.64	0.9396 (518)
Total	*Salmonella*	63654.25	140416.04	0.671 (113)
coliforms	*E. coli* O157:H7	N/A [a]	134981.58	N/A [a]

[a] *E. coli* O157:H7 was not detected in any samples for which total coliform counts were used in analysis.

Figure 2-4 Variation in faecal coliform counts with presence or absence of *E. coli* O157:H7 in water samples. Boxes represent 50% of values within the group. Whiskers represent highest and lowest values, excluding outliers, which are values that are from 1.5 to 3.0 times the length of the box from the box edge. Box lines indicate median value.

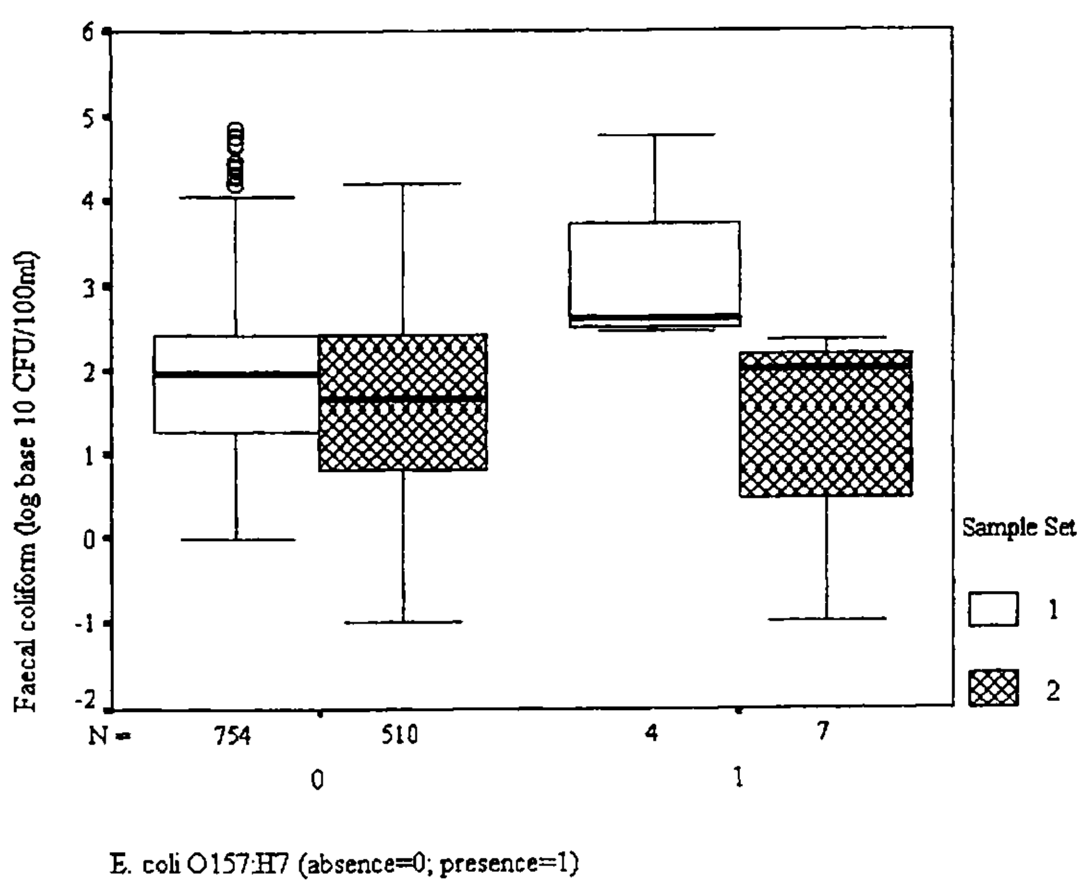


Figure 2-5 Variation in faecal coliform counts with presence or absence of *Salmonella* in water samples. Boxes represent 50% of values within the group. Whiskers represent highest and lowest values, excluding outliers, which are values that are from 1.5 to 3.0 times the length of the box from the box edge. Box lines indicate median value.

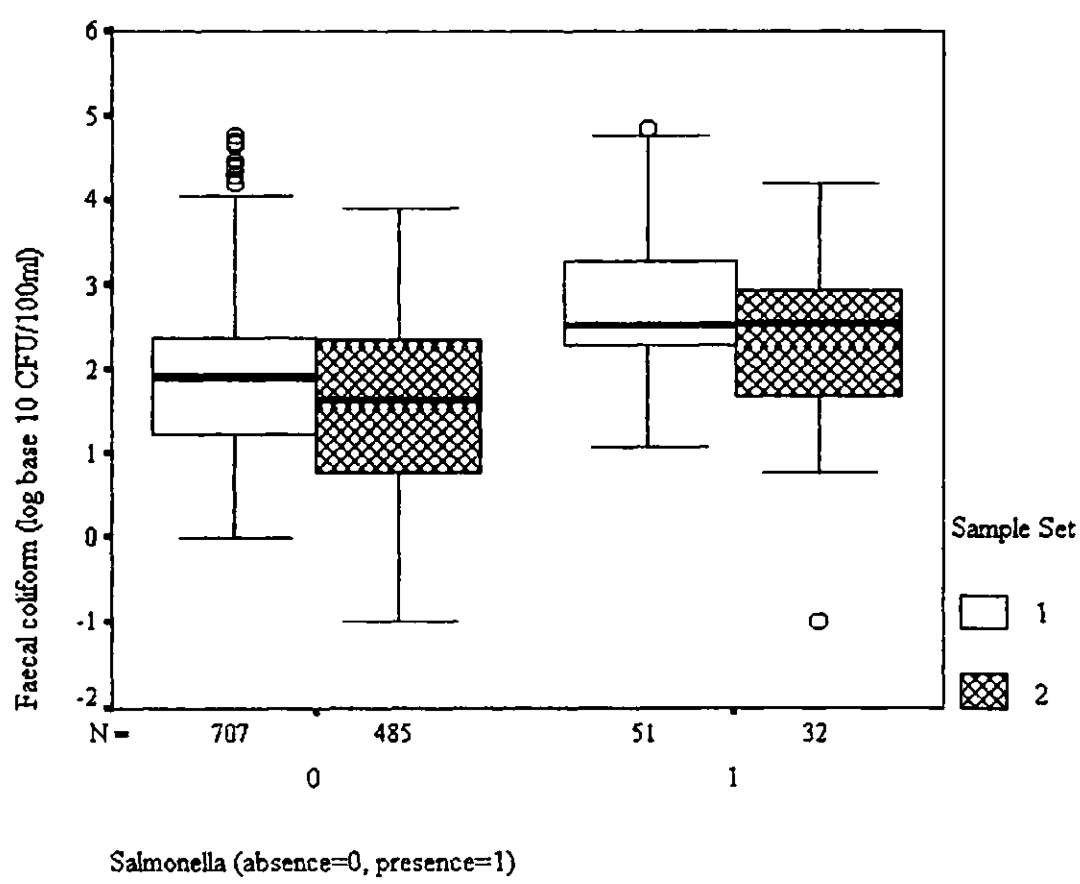


Table 2-11 Sample Set 1 measures of relative risk and predictive value. Non-significant (N/S) association was demonstrated by Fisher Exact test when 1-sided $P > 0.05$.

Water use (indicator organism)	Pathogen	Relative risk	Predictive value negative	Predictive value positive
Drinking[a]	*Salmonella*	undefined[d]	1.00	0.07
(*E. coli*)	*E. coli* O157:H7	N/S	1.00	0.01
Irrigation[b]	*Salmonella*	5.87	0.98	0.12
(faecal coliforms)	*E. coli* O157:H7	undefined[d]	1.00	0.01
Recreation[c]	*Salmonella*	4.72	0.95	0.22
(*E. coli*)	*E. coli* O157:H7	N/S	1.00	0.03
Recreation[c]	*Salmonella*	4.01	0.96	0.17
(faecal coliforms)	*E. coli* O157:H7	7.93	1.00	0.02

[a] Federal-Provincial Subcommittee on Drinking Water, 1999.

[b] Canadian Council of Ministers of the Environment, 1999.

[c] Natural Resources Service, Water Management Division, Alberta Environment, 1999.

[d] Division by zero.

Table 2-12 Sample Set 2 measures of relative risk and predictive value. Non-significant (N/S) association was demonstrated by Fisher Exact test when 1-sided $P > 0.05$.

Water use (indicator organism)	Pathogen	Relative risk	Predictive value negative	Predictive value positive
Drinking[a] (total coliforms)	*Salmonella*	N/A[d]	N/A[d]	N/A[d]
	E. coli O157:H7	N/A[d e]	N/A[d e]	N/A[d e]
Irrigation[b] (faecal coliforms)	*Salmonella*	3.59	0.97	0.11
	E. coli O157:H7	N/S	0.99	0.01
Irrigation[b] (total coliforms)	*Salmonella*	undefined[f]	1.00	0.07
	E. coli O157:H7	N/A[e]	N/A[e]	N/A[e]
Recreation[c] (faecal coliforms)	*Salmonella*	2.81	0.95	0.13
	E. coli O157:H7	N/S	0.98	0.00

[a] Federal-Provincial Subcommittee on Drinking Water, 1999.

[b] Canadian Council of Ministers of the Environment, 1999.

[c] Natural Resources Service, Alberta Environment, 1999.

[d] Could not be calculated as there were no samples for which total coliform counts were below the drinking water guideline.

[e] Could not be calculated as *E. coli* O157:H7 was not detected in any of the samples tested for total coliform counts.

[f] Division by zero.

2.4 Discussion

The presence of *E. coli* O157:H7 and some *Salmonella* serotypes in water samples suggests some degree of risk to human health if water from which those samples were obtained was consumed directly or indirectly. Among the *Salmonella* serotypes isolated were Agona, Hadar, Indiana, Typhimurium, Mbandaka, Newport, Rubislaw, Seftenberg, Thompson, Heidelberg, Schwarzengrund, Cubana, Enteriditis, and Typhimurium var. Copenhagen, which have been associated with outbreaks of disease in humans (Synott *et al.*, 1998; Faustini *et al.*, 1998; Baxter and Morton, 1985; Kramer *et al.*, 1996; Scheil *et al.*, 1998; Van Beneden *et al.*, 1998; Parish, 1998; Lehmacher *et al.*, 1995; Rushdy *et al.*, 1998; Shapiro *et al.*, 1999; Shallow *et al.*, 1999; Levy *et al.*, 1975; Anonymous, 1992; Afrasiabi and Walehhave, 1986).

The occurrence rate of *E. coli* O157:H7 in this study (0.86%) was lower than that of the study by McGowan *et al.* (1989) of 15 environmental sites in Philadelphia (3.3%, 1 of 30). It should be noted, though, that sampling dates were not published in the McGowan report, and the sample size was much smaller than the present study, hence a direct comparison between it and the present study may not be applicable.

The *Salmonella* detection rate in this study (6.04%) was similar to that in the study by Arvaitidou, *et al.* (1997) of 128 samples taken from lakes and rivers in Greece over 12 months (6.2%), but much lower than that found by Polo *et al*, (1998) in 2440 samples taken from sea (5.9%), river (58.7%), and freshwater reservoirs (14.8%) in Spain from June to September over 4 years. In the latter study the geometric mean of FC counts in river water was 12,853 CFU/100ml, much greater than that of water samples tested in this study (737.4CFU/100ml), thus the higher *Salmonella* detection rate may have been a reflection of the greater overall faecal contamination in the samples. As well, the likelihood that Polo and colleagues detected *Salmonella* in samples may have been greater as they filtered 1litre volumes prior to culturing, while the present study tested 100 ml aliquots.

Peaks in *E. coli* O157:H7 and *Salmonella* prevalence from the middle of July to the beginning of August in this study are similar to the seasonal trend of disease due to infectious agents in drinking water (June to August) (Kramer *et al.*, 1996; Levy *et al.*, 1998; Barwick *et al.* 2000). The peaks in pathogen prevalence were slightly later than that of gastrointestinal illness outbreaks associated with recreational water (June and July) in the U.S from 1993 to 1998 (Kramer *et al.*, 1996; Levy *et al.*, 1998; Barwick *et al.* 2000), and the difference may be due to the fact that the U.S. generally has warmer weather earlier than southern Alberta. The seasonal peak in *Salmonella* detection in the present study (Figures 2-2 and 2-3) is shifted one month later than that observed in freshwater in India by Sharma and Rajput (1996). In that study, peaks in *Salmonella* concentration were observed in the middle of June to the beginning of July in both years.

Storm drain, sewage treatment plant, and watershed sources appear to be contributing to the prevalence of *E. coli* O157:H7 and *Salmonella* in surface water in southern Alberta to varying degrees (Table 2-7). However, due to the small sample size of the sewage treatment plant samples, and to a lesser degree of the storm drain samples, the estimates of pathogen prevalence in these sources may not be accurate. Therefore, comparisons of prevalence among the sampling site categories should be taken with caution. That *E. coli* O157:H7 was detected from the watershed samples was not surprising as there were hoof marks in the banks at numerous sample sites, within irrigated and dryland agricultural regions (data not shown). Watershed sampling sites were often chosen where roads overpass the water system to allow easy access for workers collecting samples. Under many of these overpass locations, large populations of birds, such as cliff swallows, were common during the summer months; and may have been a factor in the occurrence of *Salmonella* in the samples (Edel *et al.*, 1976; Cizek *et al.*, 1994). In comparison to the other sampling sites within this study, there may be a greater risk of infection associated with the consumption or domestic use of water from the sampling site on the Oldman River downstream of the Battersea drain as *E. coli* O157:H7 was detected in 2 samples from that site and the prevalence of *Salmonella* in samples from this site was also high.

If the prevalence of *E. coli* O157:H7 (16.67%) and *Salmonella* (33.33%) observed in sewage treatment plant effluent samples tested in this study is typical for these plants, they may be contributing these bacterial pathogens to the water bodies that receive their discharge. If contamination from their discharge into water bodies cannot disperse well or sediment out of the water before it is used for irrigation, recreation, or residential purposes, there may be a risk of infection associated with the discharge.

The absence of *E. coli* O157:H7 in storm drain water samples suggests city surface water drainage discharge into the Oldman River has a low probability of contributing to the prevalence of this pathogen in the river. However the high prevalence of *Salmonella* in storm drain water may be a concern to public health.

BS media is considered by D'Aoust (1997) to be the preferred selective *Salmonella* plating media due to its high sensitivity and ability to detect low levels of H_2S production. As well, BS is recommended by the US Food and Drug Administration (Andrews, *et al.*, 1992) as an effective media for detection of *Salmonella* in foods.

However, as the predictive value positive of BS and BGS were 0.17 and 0.13, respectively, they were not efficient means of detecting *Salmonella* in this study.

Distinguishing suspect colonies was difficult at the beginning of this survey due to lack of laboratory experience with BGS and BS. Growth on BGS plates was frequently over-grown with spreading colonies (*Proteus* spp.). In this study, MSRV was a more specific media; its predictive value positive was 0.89. Ruiz *et al.* (1996) found *Salmonella* detection on MSRV media to be much lower in sensitivity (3.3%) than BS (100%), but in this study sensitivity of both MSRV and BS methods were high (89.2%, and 78.4%, respectively)(Table 2-8).

Detection of *E. coli* O157:H7 and *Salmonella* in samples with no faecal coliforms in 100 ml samples suggested that this pathogen can not be assumed to be absent in water samples with low coliform counts (faecal contamination was not measured). This phenomena is not unique to this study, as *Salmonella* was detected in 0.9% of the seawater samples tested by Polo *et al.* (1998) that had no measurable counts of total and faecal coliforms.

Faecal coliform counts of Sample Set 2 were poor indicators of the presence of *E. coli* O157:H7 compared to the counts of Sample Set 1(Table 2-7, Figure 2-4). Differences between the range of counts of the two sets may have been due to the diffences in water samples, as the sampling sites from Sample Set 1 had a variety of types of sites, while sites from Sample Set 2 were only irrigation drainage sites. There also may have been differences in enumeration technique; if identical samples had been tested in both laboratories it would have been helpful in revealing differences between laboratories.

Significant differences between levels of indicator organisms in pathogen-positive and pathogen-negative samples (Table 2-6) suggest that the relative magnitude of counts could be an indication of the presence of pathogens. However, the likelihood of pathogen presence in the samples could not be predicted from indicator organism counts as the logistic regression coefficients in models of indicator organism counts and pathogen presence were zero. A logistic regression coefficient describes change in the odds that a dichotomous independent variable =1 with change in an independent variable (Hosmer and Lemeshow, 1989). In this case, since the coefficients were zero, the odds of pathogen presence in a water sample were not increased with higher counts. Efstratiou *et al.* (1998) also found faecal coliform concentration was not associated with the probability of *Salmonella* presence in water when using logistic regression analysis. Models that could reliably predict the presence of pathogens would be valuable to water quality monitoring agencies, and the need for them has been noted by the World Health Organization (UNEP/WHO 1996).

Although none of the water from which pathogen-positive samples were obtained were permissible for use as drinking water, based on the guidelines, some were acceptable for recreational and, or irrigation use. While exposure is lower through these routes than through drinking, low infectious dose associated with these pathogens (Keene *et al.* 1994; Boyce, *et al.* 1995; Holcomb, *et al.* 1999; Blaser and Newman, 1982), means even accidental intake during activities such as swimming could result in infection.

Relative risk measures the strength of impact of a dichotomous independent factor on a dichotomous dependent outcome, and is commonly used in toxicology and epidemiology studies to assess causality (Gertsman, 1998). In this study, relative risk was used as a measurement of the association between pathogen presence and the status of water samples with respect to water use guidelines. For example, *Salmonella* was 4.01 times more likely to be present in water with a faecal coliform concentration above recreational water guidelines than in water concentrations below the guidelines. *Salmonella* was also 5.87 times more likely in water above the faecal coliform guidelines for irrigation water (Table 2-8). It appears then, that, for the results of this study, the irrigation guideline was more powerful than the recreational guidelines.

A review of studies of association between human health and microbial water standards by Barrell *et al.* (2000) suggests that enterococci are more strongly associated with risk to health than are total coliforms, faecal coliforms, and *E. coli*. In the absence of human health and enterococci data of the samples in this study, a test of this hypothesis could not be conducted. This would be the type of study necessary to assess risks to human health associated with indicator organisms.

Understanding pathogen prevalence is critical for the evaluation of exposure assessment in microbial risk assessments (Lammerding and Fazil, 2000). Quantitative microbial risk assessments have been used in the establishment of government-regulated water quality standards for indicator organism concentration (Macler and Regli, 1993). Data such as those presented here can be used to set water use guidelines. A model could be developed using the data that would minimise the number of samples with pathogens below certain indicator organism levels.

2.5 Conclusion

This survey of *E. coli* O157:H7 prevalence in surface water is one of the largest performed to date. This pathogen was detected in 0.86% (13 of 1520) of the samples tested over a two-year period. *Salmonella* species were isolated in 6.04% (88 of 1458) of the samples. Peaks in prevalence of both species occurred from the middle of July to early August. While there was no significant change between the years in the overall prevalence in *Salmonella*, there was an increase in the prevalence in *E. coli* O157:H7 from 1999 to 2000. The presence of *E. coli* O157:H7 and *Salmonella* serotypes associated with outbreaks of disease in humans suggests a risk to human health related to consumption of water from which these pathogens were isolated. The absence of *Salmonella* and *E. coli* O157:H7 was not always predicted in samples with concentrations of indicator organisms below the guidelines for microbial levels in water.

3 Survival of *Escherichia coli* O157:H7 and *Salmonella typhimurium* in Surface Water

3.1 Introduction

Waterborne transmission can disperse enteric pathogens over a wide area, and thus, has the potential to spread infectious diseases to many individuals (Moe, 1997; Ewald, 1994). Recent large waterborne outbreaks include an outbreak of *E. coli* O157:H7 infection in Walkerton, Ontario in 2000, in which more than 700 individuals became ill and 7 died (Kondro, 2000), and an outbreak of *S. typhimurium* infection, during which 625 became ill and 7 died in Missouri in 1993 (Kramer, *et al.*, 1996). The duration of pathogen survival in flowing surface water is a critical factor in the extent of the potential zone of contamination, and number of individuals put at risk of infection. Persistence also increases the time during which standing water in contained water bodies (lakes, reservoirs) containing these bacteria can be a health hazard.

While bacterial survival models are not common for waterborne bacteria (Utzet *et al.*, 1996), they are commonly used in food microbiology to predict shelf-life, and the effects of pathogen control measures for foods (Griffiths, 1994; van Gerwen and Zwietering, 1998). The three components of bacterial survival curves are the shoulder, linear decline, and tail phases (Xiong *et al.* 1999; Whiting, 1993; Cerf, 1977). The microbial dynamics that may have a role in the characteristics of each phase are presently not well understood. Many predictive models exist that use standard curve equations to develop models of responses of specific bacteria, in a given substrate, under fixed environmental conditions (Abee and Wouters, 1999; Whiting and Buchanan, 1997).

Some studies have considered the survival of pathogenic bacteria in water as it varies with water temperature, species of pathogen, and concentration of autochthonous micro-organisms (indigenous to the ecosystem)(Flint, 1987; McCambridge and McMeekin, 1980; Wang and Doyle, 1998; Rhodes and Kator, 1988; McCambridge and McMeekin, 1981; Warburton *et al.,* 1994).

The hypotheses for this study were that survival of *E. coli* O157:H7 and *S. typhimurium* in surface water are effected by various environmental conditions, and that survival would vary between the 2 pathogen species and between waterborne strains and strains isolated from other sources. Environmental conditions considered included temperature, turbidity, autochthonous bacteria, and initial concentration of pathogen inoculum. As well, the differences in response between the two species, and between bacteria of these species isolated from water and ones isolated from the fauna of vertabrate hosts were examined. Characteristics of survival curve components examined in this study include overall survival duration, presence of a shoulder phase, duration of tail and decline phases, and average acceleration during the decline phase. Acceleration during the decline phase was developed as a novel parameter to address the issue of modelling non-linear decline.

3.1 Methods and Materials

3.2.1 Water Collection and Characterisation

The study is comprised of two experiments. The first observed survival in filtered and non-filtered water labelled Waters A and B collected from 2 different sites. Bacterial counts were made on duplicate Trypticase Soy Agar (TSA) plates. In the second experiment growth from non-filtered water from 5 sites B through F was enumerated on MacConkey agar in duplicate. Water A was collected in February 2000, and Waters B through F were collected in July 2000; all collection sites are briefly described in Table 3-1 and shown in Appendix 4. All water samples were collected in sterile 2 L flasks, triple-rinsed and held 20 cm below the surface, upstream of the foothold, and transported in chilled containers. Duplicate turbidity readings (optical density (OD) at 600 nm) were taken of Waters B through F. Estimates of autochthonous bacteria concentration in the non-filtered water collected from each site were obtained from plate counts on TSA or Luria-Bertani (LB) agar. Samples of 0.1 ml were spread on the agar, and incubated at 37^{O} C for 24 ±2h.

Table 3-1 Descriptions of sites from which water was collected for survival study.

Water label	Water collection site location	Site characteristics
A	Olin Creek, 15 km upstream of the Oldman River Reservoir bottom release	Very clear water; rock bottom; shallow, high flow.
B	Little Bow River, near the mouth	Very turbid water, no cattle present
C	Little Bow River, 8 km downstream of Travers Reservoir outflow	Turbid water, with many macrophytes and cattle access
D	Little Bow River approximately 200m downstream of the Travers Reservoir outflow	Very clear water, with some macrophytes and cattle access
E	Lethbridge Northern Irrigation District distribution intake from the Oldman River	Very clear water, no macrophytes or livestock access
F	Little Bow River, 2 km downstream of Sorgaard drain and cattle feeding operations	Very turbid water, cattle access

3.2.2 Isolation of Nalidixic Acid-Resistant Mutant Pathogenic Strains

Broth cultures of *S. typhimurium* phage type 110b (LCDC# SA994692) and *E. coli* O157:H7 phage type 31 (LCDC# EC990850), a laboratory strain of *S. typhimurium*, phage type Z30 (ATCC 14028), and a bovine isolate of *E. coli* O157:H7 were swabbed onto TSA (Difco, Detroit), and incubated overnight at 37°C. Lawns of bacterial growth were swabbed and streaked onto TSA containing a nalidixic acid concentration of 200 μg/ml and incubated 24 ±2 h at 37°C. Single colonies were streaked onto TSA with nalidixic acid (200 μg/ml) and incubated at 37°C for 24 ±2 h. One day prior to experiments, colonies were inoculated into 10 ml of BHI (Difco, Detroit) or Trypicase Soy broth (TSB, Difco, Detroit) with 0.02 ml of 100 mg /ml-nalidixic acid, and then incubated at 37°C, shaken at 150 rpm. (Henceforth, reference in this text to detection media used in this survival study will be assumed to include nalidixic acid at a concentration of 200 μg/ml).

3.2.3 Preparation and Organisation of Samples

To test for the presence of nalidixic acid-resistant organisms in the source water, 0.1 ml of the non-filtered water from each site was cultured in TSB (37 °C, 150 rpm for 24 ± 2 h) and plated on TSA.

Half of the volume of Waters A and B were filtered through 0.2 μm pore fibreglass filter units (Nalgene, Rochester), and poured into sterile flasks or bottles. The other half of Waters A and B, and Waters C through F were not filtered.

Turbidity (OD 600nm) of *S. typhimurium* and *E. coli* O157:H7 culture broths was measured. Cells were harvested by centrifugation (4000 X *g*, 10 min). To prevent nutrient carry-over from the culture to the water inocula, cells were washed in 0.1M phosphate buffered saline (PBS) 3 times. The washed culture was diluted into PBS and the required volume to obtain approximately 10^3 CFU/ml was added to the water samples. Following pathogen inoculation into the water sample the samples were mixed with a magnetic stirrer for at least 1 minute and decanted in 125 ml to 200 ml volumes into sterile 250 ml glass bottles. Each bottle represented an experimental sample of water from one site, with one strain of inoculated bacteria, held at either $10^{\circ}C$ or $20^{\circ}C$. Multiple samples of the same conditions, comprising a sample set, were tested. Four samples were used in each of the 16 sample sets of the water from Site A (4 strains, 2 temperatures, 2 filtration treatments). Three samples were grown in each of the 8 sample sets of water from Site B (2 strains, 2 temperatures, 2 filtration treatments), and the 4 sample sets of water from Sites C through F (2 strains, 2 temperatures). Water was held at $10^{\circ}C \pm 3^{\circ}C$ in a refrigerator, or at $20^{\circ}C \pm 3^{\circ}C$ in a cool storage room. Figures 3-1, 3-2 and 3-3 illustrate the sample organisation.

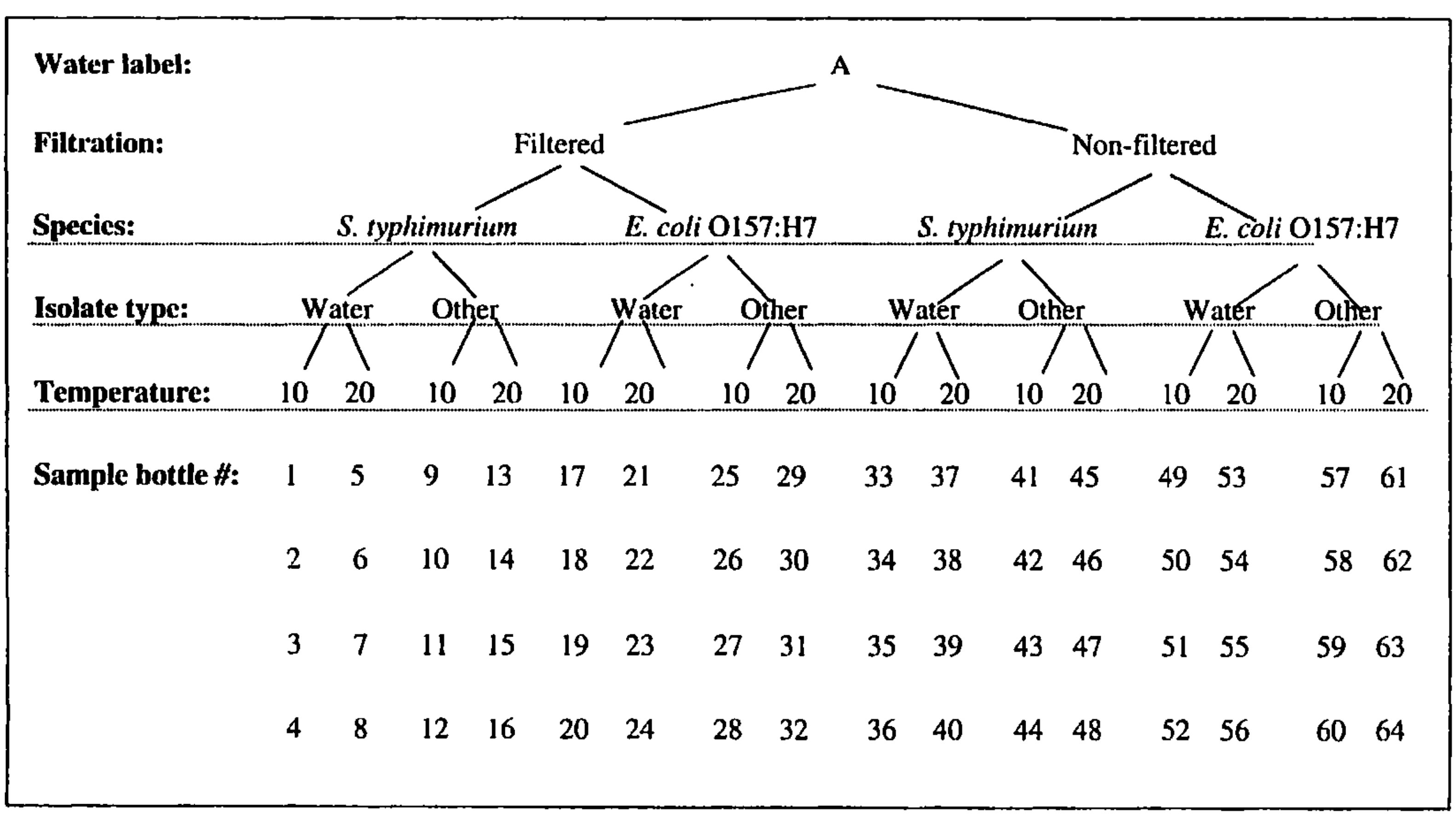


Figure 3-1 Schematic outline of filtration, temperature, and inocula of samples of Water A.

Figure 3-2 Schematic outline of filtration, temperature, and inocula of samples of Water B plated on TSA.

Water label:	B							
Filtration:	Filtered				Non-filtered			
Species:	*S . typhimurium*		*E. coli* O157:H7		*S. typhimurium*		*E. coli* O157:H7	
Temperature:	10	20	10	20	10	20	10	20
Sample bottle #:	65	68	71	74	77	80	83	86
	66	69	72	75	78	81	84	87
	67	70	73	76	79	82	85	88

Figure 3-3 Schematic outline of temperature and inocula of samples of Waters B through F plated on MacConkey agar.

Water label:	B, C, D, E, or F			
Species:	*S. typhimurium*		*E. coli* O157:H7	
Temperature:	10	20	10	20
Sample:	89	92	95	98
	90	93	96	99
	91	94	97	100

3.2.4 Enumeration

Bottles were mixed by inversion or swirling and a 0.1 ml aliquot was withdrawn with a pipette and spread on duplicate solid media. Following inoculation plates were placed into a 37°C incubator for approximately 1h, then inverted and incubated for 16 h ± 1 h. Solid media were kept in opaque containers and were warmed at 37°C for 1h prior to use. Colony counts were taken using a pen counter (Scienceware F37862-000, Pequannock).

Survival of inocula in samples of Waters A and B was estimated from growth on TSA. Samples of Waters B through F were also cultured on TSA from Day 0 to Day 3. Growth in TSB and on TSA of nalidixic acid-resistant autochthonous bacteria was observed in water sampled from one site. Thus, this water was not included in the study. At this point, the survival experiment using Water A was completed, as were the first 3 days of the survival experiments using Waters B through F. From this point on in the study, MacConkey agar was used as an alternative to TSA. A comparison study was performed to assess any difference between growth of the pathogens on MacConkey agar and TSA. The counts obtained from TSA in the first 3 days were then transformed to counts equivalent to what would likely have been obtained from MacConkey agar.

3.2.5 Survival Curve Parameters and Factors

The survival curves described had three distinct phases of pathogen response to environmental stresses: the shoulder, decline, and tail phases. Five dependent curve parameters were used to characterise these phases. These were: presence or absence of a shoulder phase, duration of the decline phase, average acceleration of the decline, duration of the tail phase, and overall duration of the survival curve. A typical plot, with the components of survival curves, is illustrated in Figure 3-4. Summaries of the independent factor variables and dependent parameter variables are given in Tables 3-2 and Table 3-3, respectively.

Percent of initial concentration of inoculum (C_0) was plotted against time for each sample using mean sample concentration (CFU/0.1 ml). Linearity was assumed between sampling days. The last time the sample average count was 100% of initial concentration, C_0 was interpolated and was assumed to be the beginning of the decline phase, $t_{0\ decline}$. If, for a given sample, $t_{0\ decline}$ was greater than zero, a shoulder phase was said to be present in the survival curve.

Following visual inspection of the survival curves (data not shown), the boundary conditions were established for the beginning of the tail phase ($t_{0\ tail}$). In most survival curves of pathogens in non-filtered samples $t_{0\ tail}$ was the first time when the count was 6.5% of C_0, and for filtered samples, it was when the count was 30% of C_0. The value of $t_{0\ tail}$ for each sample was interpolated from the data.

The time between the beginning of the decline phase ($t_{0\ decline}$) and $t_{0\ tail}$ was designated as the duration of the decline phase, abbreviated in this text as $\Delta t_{decline}$.

The last time the sample average concentration was 0.1% of C_0 was interpolated from the data, and this measure of the total survival duration was abbreviated as $t_{0.1}$. This is equivalent to a 3 decimal reduction in concentration, D3, but $t_{0.1}$ was felt to be a more descriptive term. While the inclusion of the tail phase duration is inferred in the duration of $t_{0.1}$, D3 may not always include the tail phase. Duration of the tail phase for each sample was calculated as the time between $t_{0\ tail}$ and $t_{0.1}$ and was abbreviated as Δt_{tail}.

The sampling days closest to $t_{0\ decline}$ and the beginning of the tail phase were determined. From visual inspection, it appeared that the decline phase for most samples was non-linear, thus second-degree polynomials were fitted through the points starting on the sampling day closest to $t_{0\ decline}$ and ending on the sampling day closest to $t_{0\ tail}$ of each plot (Excel version 9.0). The average acceleration of a function is defined as its second derivative, thus the average acceleration of decline during the decline phase, *A*, of the second-degree polynomial decline curve, was estimated as 2 times the second-degree term coefficient of the polynomial. As the second derivative of a linear function is also the curvature, the assumption of non-linearity of the decline phase was tested. When the polynomial curve was formed by only 3 points and was not close to linear, the curve was considered to not be a best-fit line, or if the boundary condition for $t_{0\ tail}$ was not fulfilled, *A* was not calculated.

Figure 3-4 Example of survival curve elements. The beginning of decline phase was defined as the last time the mean sample concentration was 100% of C_0. The beginning of tail phase was defined as the first time mean sample concentration was 6.5% of C_0 in non-filtered samples, and 30% of C_0 in filtered samples. The end of tail phase was defined as the last time mean sample concentration was 0.1% of C_0.

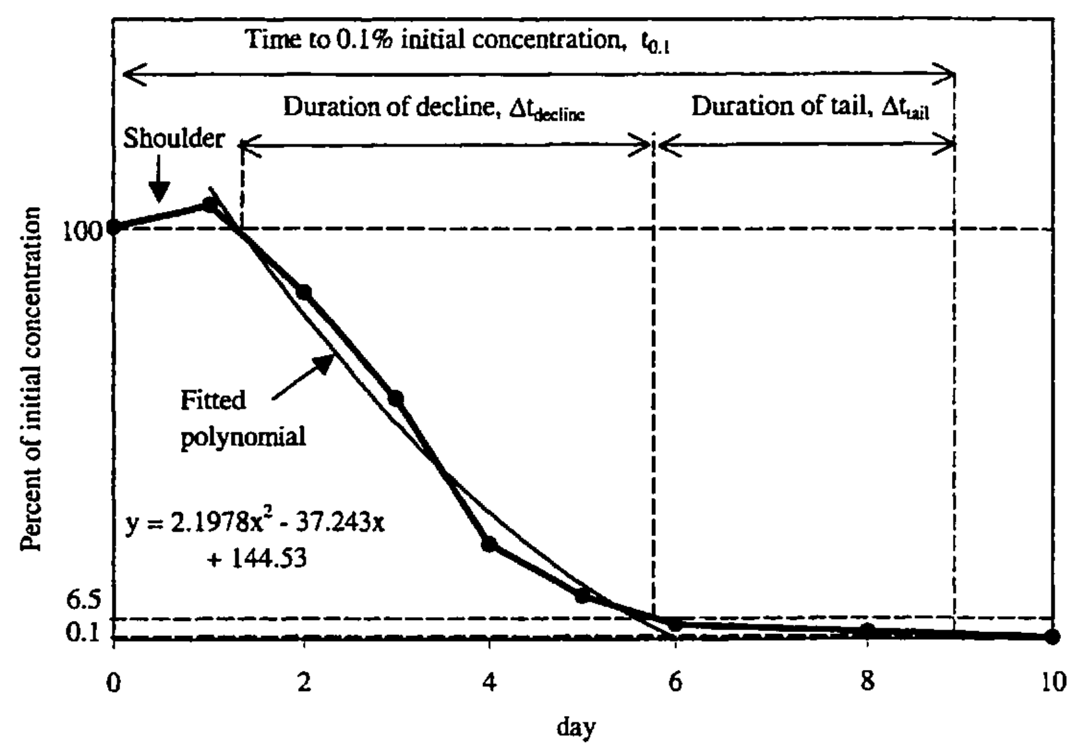


Table 3-2 Independent variables examined in study.

Factor	Description (values)	Application
Species	dichotomous, nominal (*S.typhimurium, E. coli* O157:H7)	all
Isolate type	dichotomous, nominal (water, other)	Water A
Temperature	dichotomous, interval (10°C, 20°C)	all
Filtration	dichotomous , nominal (yes, no)	Waters A and B
Autochthonous bacteria level	continuous, ratio	all
Turbidity	continuous, ratio	Waters B to F
Initial concentration	continuous, ratio	all

Table 3-3 Dependent curve parameter variables used in study.

Parameter	Unit	Description	Equation
$t_{0.1}$	d	Time at which average count is 0.1% of initial	none
Δt_{tail}	d	Duration of tail phase	$t_{0.1} - t_{0\ tail}$
$\Delta t_{decline}$	d	Duration of decline phase	$t_{0\ tail} - t_{0\ decline}$
A	% / d^2	Average acceleration during decline phase	$2a_1$
Shoulder	none	Presence or absence of shoulder phase	none

3.2.6 Statistical Analysis

The mean of duplicate plate counts for each sample was used in all statistical analysis. All statistical analyses were performed on the Statistical Package for the Social Sciences (SPSS, Chicago).

Bivariate correlation analysis was performed to test for a significant relationship between autochthonous bacteria concentration and turbidity. One-sample means test (test value, mean =0) of all values of *A* was used to assess if the assumption of non-linear decline phase (outlined above) was valid.

In order to determine which factors had no significant effect on curve parameter data two-way univariate ANOVA tests were performed (Iversen and Norpoth, 1987). Categorical independent variables (temperature, species, source of isolate, autochthonous bacteria concentration, and filtration) were entered as fixed factors, then significant factors ($P < 0.05$) were identified. If a factor had no significant effect on a parameter, the parameter values were assumed to be homogeneous within the factor category (Bach *et al.*, 1999). Based on this assumption, curve parameter data within non-significant factors of samples were pooled in order to increase the number of samples that could be tested for association to autochthonous bacteria concentration and turbidity (non-parametric Spearman's correlation coefficients). Conversely, if a factor was determined by the two-way ANOVA results to have a significant effect on a parameter, the parameter values within the categories of that factor were not pooled and statistical analysis was performed on each category separately.

Spearman's rank test was also used to measure the correlation between C_0 of inoculum and curve parameters.

Pearson χ^2 was calculated for 2X2 tables to test effects of species, temperature, filtration, and source of isolate on presence/absence of shoulder. Logistic regression techniques were used to test for an increase in the probability of shoulder phases with increases in initial inoculum concentration.

3.3 Results

3.3.1 Water Characterisation

In Table 3.4 characteristics of the water prior to inoculation are listed. Generally higher plate counts were obtained from water with higher turbidity, however, no association between turbidity and autochthonous bacteria counts could be inferred (regression $R^2 = 0.377$; $t = 1.349$; $P = 0.2702$). Turbidity in the samples, then, was not a reflection of indigenous bacterial concentration.

Table 3-4 Characteristics of surface water prior to inoculation. Characteristics of Waters A and B were measured prior to filtration and inoculation. Mean plate counts were assumed to measure autochthonous bacteria in the samples.

Water label	Mean plate count (CFU/0.1ml) (*n*=2)	Mean turbidity, OD 600nm (*n*=2)
A	0 [a]	Not tested
B	55.5 [b]	0.0095
C	91.5 [b]	0.0215
D	12 [b]	0.0030
E	77 [b]	0.0020
F	79 [b]	0.0255

[a] indicates TSA counts, with no nalidixic acid in media

[b] indicates LB counts, with no nalidixic acid in media

3.3.2 Media and Enumeration Techniques

Paired *t*- tests indicated that there was a significant difference ($P < 0.05$) between duplicate plate counts of 5 of 144 (3.5%) samples of the filtered Water A (data not shown). The media comparison study results showed significantly lower mean counts of *S. typhimurium* (2-tailed *t*-test, $t = -3.250$; $P = 0.007$; df=7) and *E. coli* O157:H7 ($t = -3.548$; $P = 0.003$; df=9) on MacConkey agar than those on TSA (data not shown). Consequently, pathogen survival measured from MacConkey plate counts and TSA plates could not be pooled.

3.3.3 Survival Curve Parameter Data

Statistical measures of the curve parameter data from the experiments are presented in Appendices 5 and 6. Graphs of time versus mean percent of C_0 for each sample are in Appendices 7 through 50, decline phase plots for each sample are in Appendices 51 through 94. Decline phases, as fit by second-degree polynomials were not straight lines. One-way *t*-tests using zero as the test value, suggest the mean curvature of the decline phases of all samples was non-zero (Table 3-5).

Table 3-5 Test results of the assumption of non-linearity in decline phase curves. Acceleration (A) measured curvature of decline phase of samples, thus, the t-test value for mean $A = 0$.

Samples	Mean A	t	df	P
Waters A,B (TSA)	-0.379	-2.135	82	0.036
Waters B through F (MacConkey agar)	1.740	2.765	59	0.008

3.3.4 Variation in Survival Curve Parameters with Categorical Factors

Almost every survival curve parameter differed with a unique set of factors (Tables 3-6 and 3-7). While the time parameters ($t_{0.1}$, Δt_{tail}, $\Delta t_{decline}$) differed with filtration in Waters A and B plated on TSA, it did not influence the acceleration during decline, *A*. Also, of note is the fact that there was variation in all survival curve parameters of the samples plated on MacConkey agar with water label (Table 3-7). The variation in Δt_{tail} among the collected water is shown in Figure 3-5. As there was no significant difference between parameter values of the two species (Table 3-7), parameter values for both *E. coli* O157:H7 and *Salmonella* in Waters B through F were pooled prior to further analysis.

Table 3-6 ANOVA results testing for differences in survival parameters of pathogens in Waters A and B (counts on TSA) due to site, temperature, filtration, species, and source of isolate. SS= sums of squares, MS = mean square. If $P \geq 0.05$ for a factor, parameter values within the categories of that factor were pooled prior to further analysis of effects.

Parameter	Stat.	Water label [a]	Temperature (°C)	Filtration	Species	Isolate source
$t_{0.1}$	SS	854.67	108.89	74935.82	130.47	2563.59
(d)	df	1	1	1	1	1
	F	31.219	3.978	2737.227	4.766	93.642
	P	0.000	0.052	0.000	0.034	0.000
$\Delta t_{tail,}$	SS	188.73	794.88	41594.79	254.43	1008.60
(d)	df	1	1	1	1	1
	F	2.697	11.359	594.410	3.636	14.413
	P	0.108	0.002	0.000	0.063	0.000
$\Delta t_{decline}$	SS	186.52	141.83	8821.68	229.94	5.27
(d)	df	1	1	1	1	1
	F	1.588	1.207	75.092	1.957	0.045
	P	0.212	0.276	0.000	0.167	0.833
A	SS	1.21	0.469	3.63	15.02	2.58
($\%/d^2$)	df	1	1	1	1	1
	F	0.777	0.302	2.336	9.677	1.660
	P	0.382	0.585	0.132	0.003	0.203

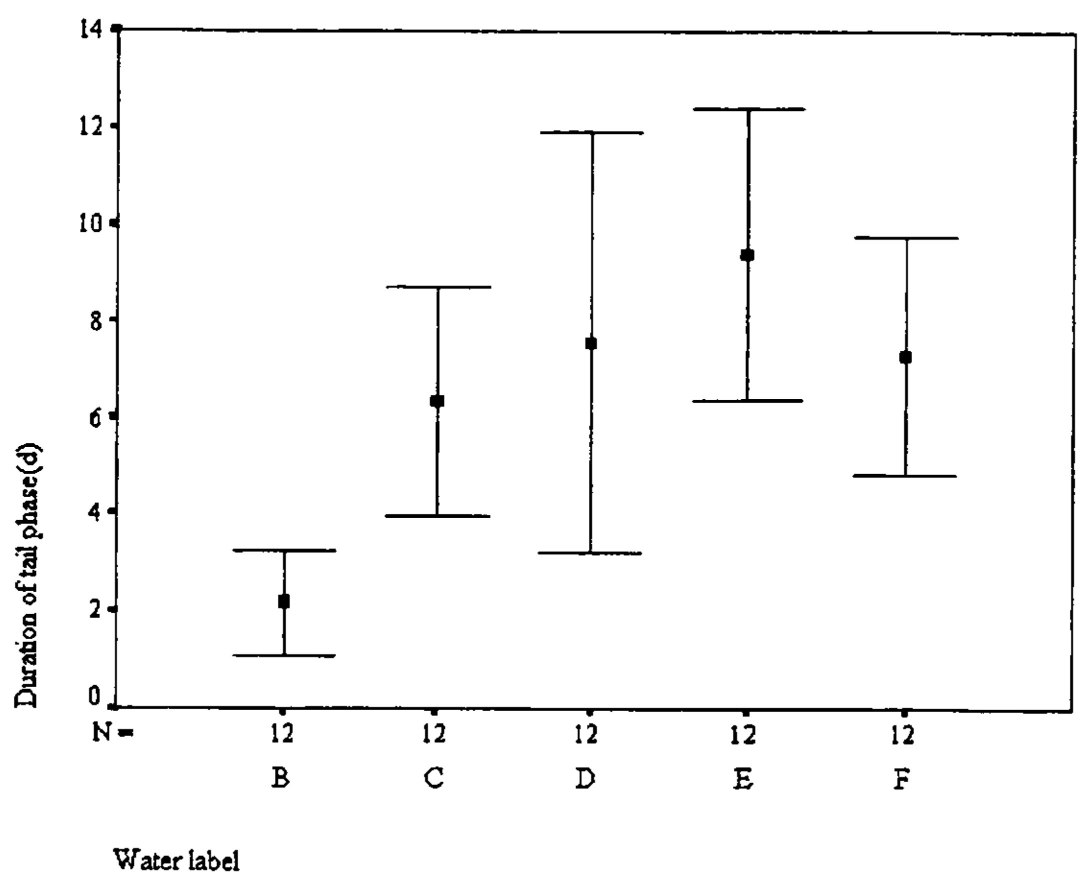
[a] Autochthonous bacteria level and turbidity were linked to water labels; i.e. each water from each label (A through F) had one autochthonous bacterial concentration and one turbidity measurement .

Table 3-7 ANOVA results testing for differences due to site, temperature, and species in survival curve parameters for pathogens in Waters B through F (counts on MacConkey agar). SS= sums of squares, MS = mean square. If $P \geq 0.05$ for a factor, parameter values within the categories of that factor were pooled prior to further analysis of effects.

Parameter	Statistic	Water label [a]	Temperature (°C)	Species
$t_{0.1}$	SS	507.97	147.96	40.31
(d)	df	4	1	1
	MS	126.99	147.96	40.31
	F	6.399	7.456	2.031
	P	0.000	0.009	0.162
Δt_{tail}	SS	350.58	2.56	46.48
(d)	df	4	1	1
	MS	87.65	2.56	46.48
	F	5.265	0.154	2.792
	P	0.002	0.697	0.103
$\Delta t_{decline}$	SS	52.63	177.02	0.047
(d)	df	4	1	1
	MS	13.16	177.02	0.047
	F	21.948	295.276	0.078
	P	0.000	0.000	0.781
A	SS	338.74	166.67	81.11
($\%/d^2$)	df	4	1	1
	MS	84.69	166.67	81.11
	F	8.218	16.174	7.871
	P	0.000	0.000	0.008

[a] Autochthonous bacteria level and turbidity were linked to water labels; i.e. each water from each label (A through F) had one autochthonous bacterial concentration and one turbidity measurement .

Figure 3-5 Variation in Δt_{tail} among Waters B, C, D, E, F. Error bars indicate 95% confidence interval, and markers indicate mean value.



3.3.5 Association between Parameters and Autochthonous Bacteria and Turbidity

Turbidity of the water had a significant negative association with $\Delta t_{decline}$ for samples held at 20^0C (Table 3-8, Figure 3-6), while autochthonous bacteria concentration was not related to any curve parameters for samples plated on MacConkey agar (Table 3-9).

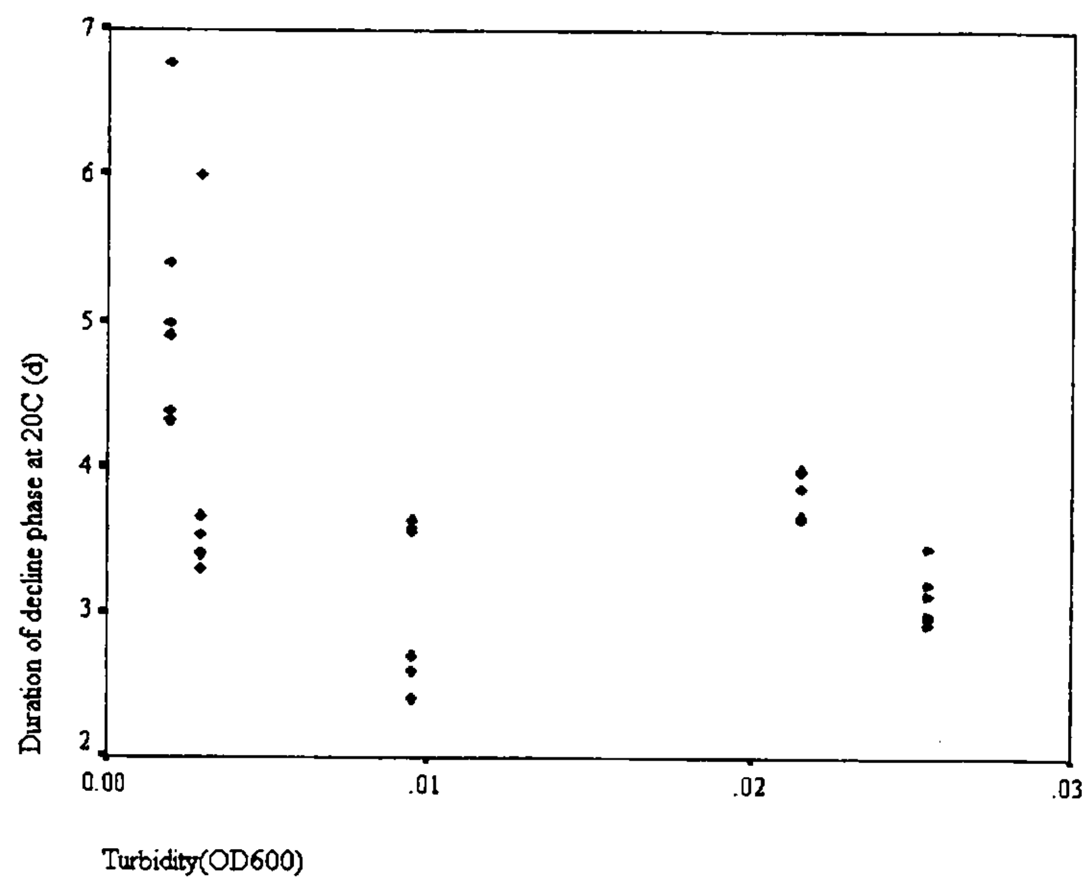
Table 3-8 Spearman correlation between turbidity and survival curve parameters.

Parameter	Temperature (°C)	Coefficient	*n*	*P*
$t_{0.1}$ (d)	10	-0.229	30	0.224
	20	-0.114	30	0.547
Δt_{tail} (d)	10	-0.087	30	0.647
	20	-0.063	30	0.742
$\Delta t_{decline}$ (d)	10	-0.302	30	0.104
	20	-0.561	30	0.001

Table 3-9 Spearman correlation between autochthonous bacteria concentration and survival curve parameters.

Parameter	Temperature (°C)	Coefficient	*n*	*P*
$t_{0.1}$ (d)	10	0.272	30	0.145
	20	0.180	30	0.342
Δt_{tail} (d)	10	0.267	30	0.154
	20	0.155	30	0.413
$\Delta t_{decline}$ (d)	10	0.267	30	0.154
	20	0.139	30	0.464

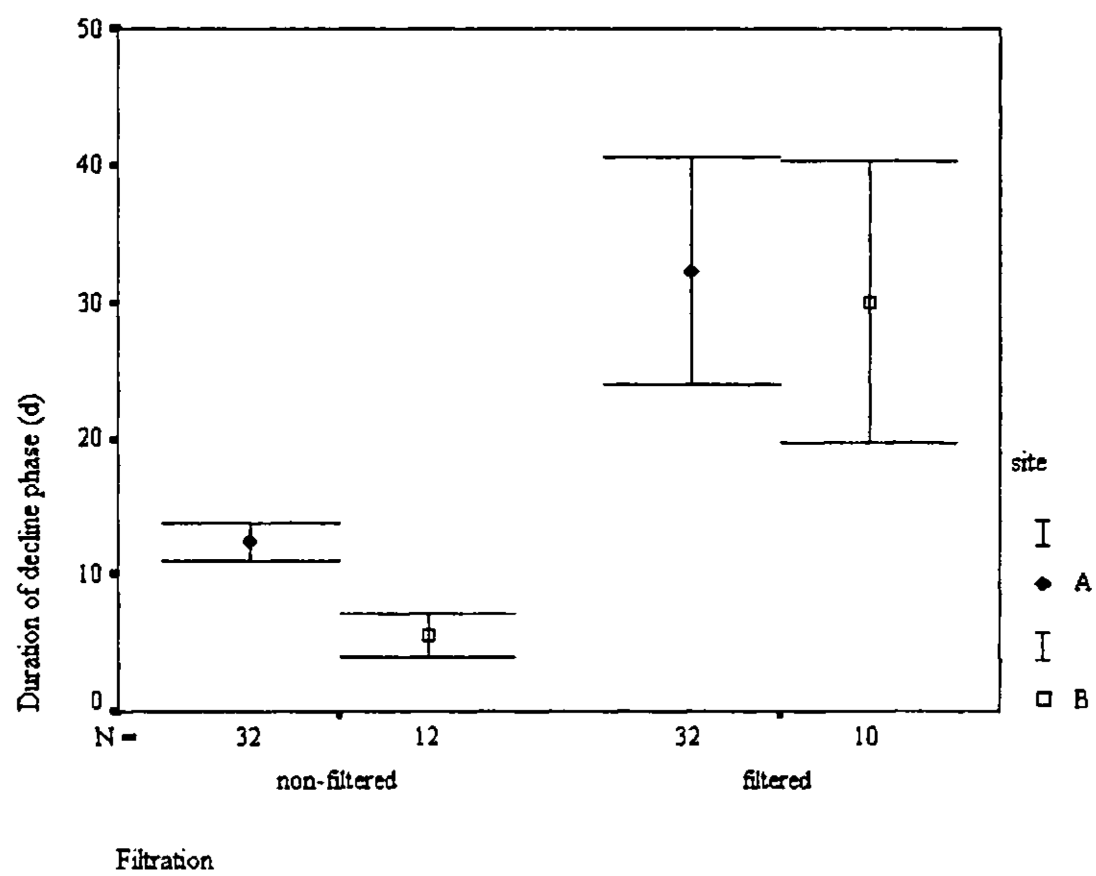
Figure 3-6 Decrease in $\Delta t_{decline}$ with increase in turbidity for *E. coli* O157:H7 and *Salmonella* in Waters B through F.



3.3.6 Effect of Filtration on Curve Parameters

The time measurements $t_{0.1}$, Δt_{tail}, and $\Delta t_{decline}$ differed with filtration treatment (Table 3-6). The mean of pooled values of $\Delta t_{decline}$ in filtered water was 31.72 days, while the mean in non-filtered water was 10.46 days ($t = 6.385$, df=44.9, $P = 0.000$). Interestingly, the mean $\Delta t_{decline}$ values in filtered Water A were significantly higher than in non-filtered Water A (mean $\Delta t_{decline}^{filtered} = 32.27$ days, $\Delta t_{decline}^{non\text{-}filtered} = 12.33$ days, $t = 4.845$, df =32.8, P =0.000) even though non-filtered Water A did not have autochthonous bacteria (Table 3-4). Figure 3-7 shows the difference due to filtration in Water A is smaller, but similar to the difference in Water B.

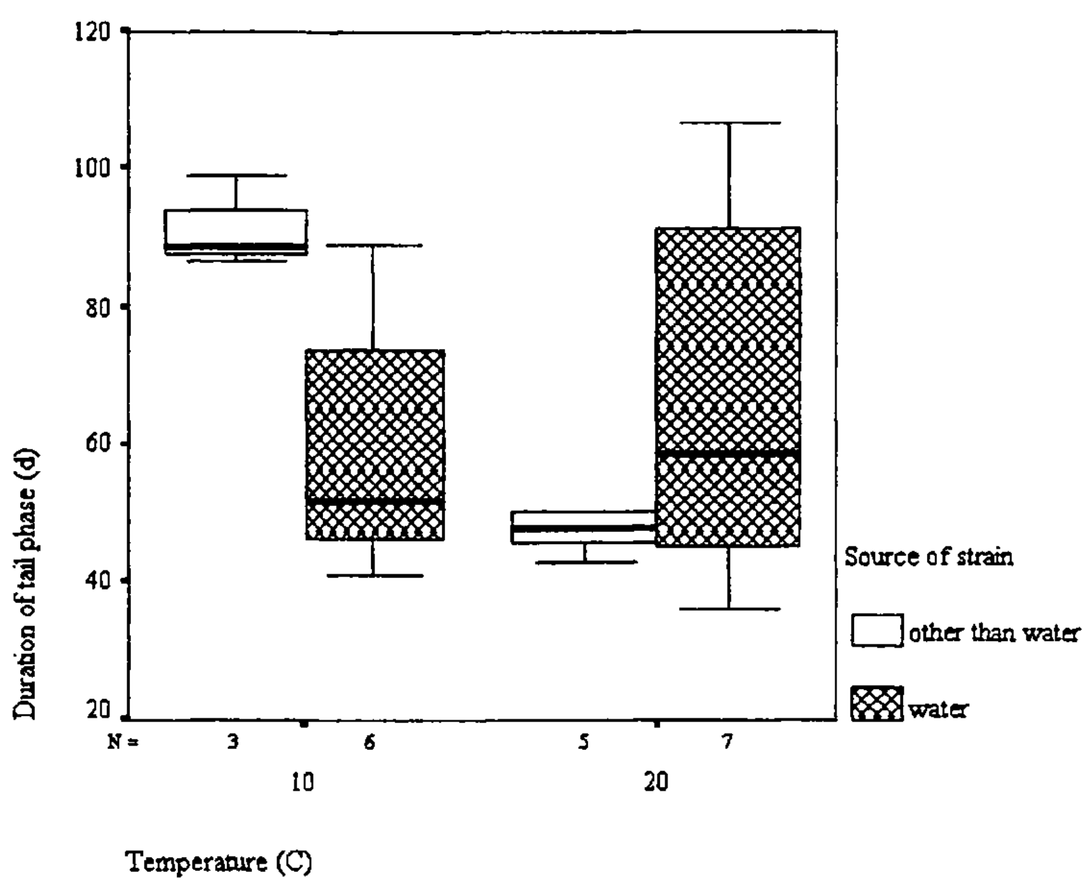
Figure 3-7 Variation in $\Delta t_{decline}$ with filtration for *E. coli* O157:H7 and *Salmonella* in Waters A and B. Water A had no autochthonous bacteria, while Water B had 55.5 CFU/0.1ml. Error bars indicate 95% confidence interval, and markers indicate mean value.



3.3.7 Variation in Parameters with Pathogen Species and Source of Isolate

The only parameter that differed significantly between the pathogen species was *A* (Tables 3-6 and 3-7). While on average, *S. typhimurium* in Waters A and B dropped in counts at the onset of the decline phase (mean A = -0.840 %/d^2), *E. coli* O157:H7 did not (mean A = 0.0714 %/d^2), and the difference in acceleration during decline was significant (t =-2.662, df = 81, P =0.009). Significant differences in Δt_{tail} existed between waterborne and isolates from other sources in filtered Water A at 10 °C (F = 8.236, df =1, P =0.024), however no significant difference existed in the filtered Water A at 20 °C (F = 0/019, df =1, P =0.894) (Figure 3-8).

Figure 3-8 Variation in Δt_{tail} with source of strain and temperature in filtered Water A. Boxes represent 50% of values within the group. Whiskers represent highest and lowest values, excluding outliers, which are values that are from 1.5 to 3.0 times the length of the box from the box edge. Horizontal box lines indicate median values. The median and 50% of values of Δt_{tail} was greater at 10^0C than at 20^0C for strains not isolated from water, but this same trend was not seen in the waterborne strains.



3.3.8 The Effect of Temperature on Survival Curve Parameters

Temperature had a strong influence on all continuous curve parameters except for Δt_{tail} for pathogens in Waters B through F (Table 3-7). In general, $t_{0.1}$ and $\Delta t_{decline}$ are less when pathogens were held at 20^0C than when held at 10^0C (Table 3-10, Figure 3-10). However, mean values of $t_{0.1}$ were greater in Waters E and F at 20^0C than when held at 10^0C (Table 3-10), suggesting the possibility of some interactive effect between temperature and some other factor associated with the water samples may have influenced the ability of the pathogens to persist.

Figure 3-11 shows a comparison of the mean $\Delta t_{decline}$ values for *E. coli* O157:H7 and *S. typhimurium* (assuming species has no effect on $\Delta t_{decline}$, see Table 3-5) between filtered and non-filtered Water B at 10 and 20 °C, and indicates that pathogens benefit from the higher temperature when no bacteria are present (filtered water), but are at a disadvantage in non-filtered water. A significant difference (P =0.000) existed between $\Delta t_{decline}$ at 10 °C (mean = 7.77 days) and 20 °C (mean = 3.20 days) in the non-filtered Water B. However, the same was not true in filtered Water B at 10 °C (P =0.439; mean = 26.3 days) and 20 °C (mean = 35.5 days).

Table 3-10 Variation in survival curve parameters with temperature and water label for *E. coli* O157:H7 and *Salmonella*. All values are means, *n* in parentheses.

Parameter	Temp. (°C)	B	C	D	E	F
$t_{0.1}$ (d)	10	9.93 *(6)	18.30 *(6)	14.74(6)	16.31(6)	10.44(6)
	20	5.56(6)	7.17(6)	10.98(6)	17.07(6)	12.93(6)
$\Delta t_{decline}$ (d)	10	6.73 *(6)	8.96 *(6)	6.52 *(6)	8.50 *(6)	5.50 *(6)
	20	3.09(6)	3.81(6)	3.88(6)	5.14(6)	3.11(6)
A (%/d^2)	10	-0.33 *(6)	0.24(6)	0.78 *(6)	-2.20(6)	1.87(6)
	20	3.52(3)	-0.56(6)	13.32 (6)	0.63(6)	4.87(6)

* Significant difference between temperatures at a level of 0.05.

Figure 3-9 Variation in $\Delta t_{decline}$ with temperature for *E. coli* O157:H7 and *Salmonella* in Waters B through F. Outliers shown (O) are those values between 1.5 and 3 times the box length from the top edge of the box, while extreme values (ж) are those that are more than 3 box lengths from the top of the box.

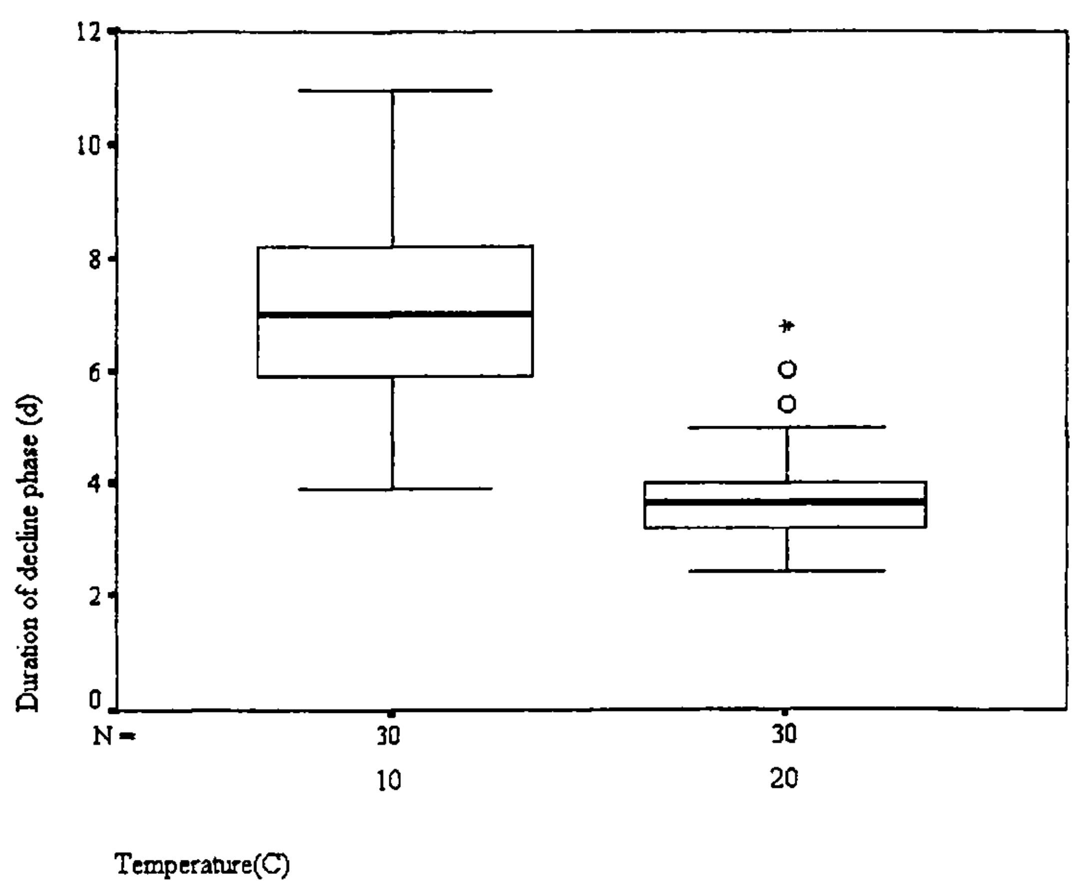
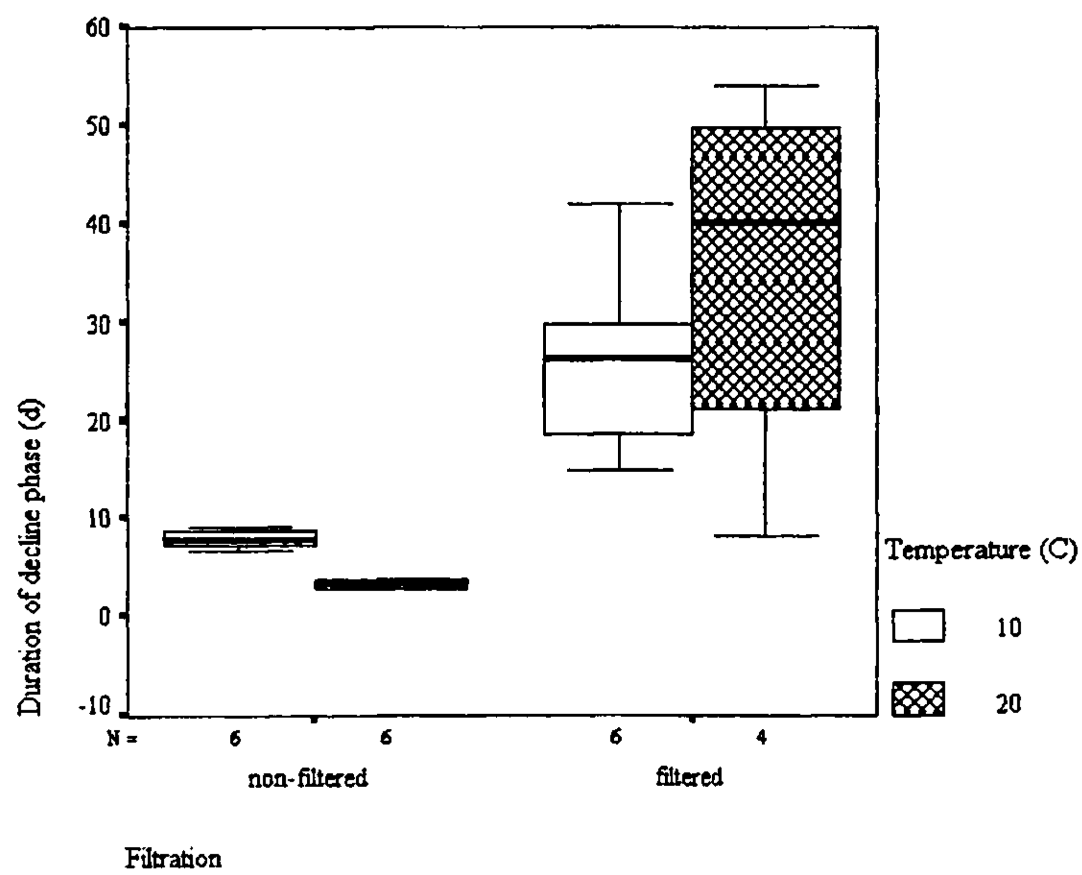


Figure 3-10 Variation in $\Delta t_{decline}$ with temperature and filtration for *E. coli* O157:H7 and *S. typhimurium* in Water B. Whiskers represent highest and lowest values, excluding outliers, which are values that are from 1.5 to 3.0 times the length of the box from the box edge. Horizontal box lines indicate median values.



3.3.9 Relationship Between Initial Concentration and Curve Parameters

The range and mean C_0 varied among water samples (Figure 3-12). Due to laboratory error the water samples were not inoculated equally, and as a result, Waters D, and to a lesser extent, E, and F were inoculated with a greater concentration than Waters B and C. Hence, testing for association was performed within each category of water label. Time to 0.1% of C_0 decreased with higher values of C_0 of pathogens in Waters E and F (Table 3-11, Figure 3-13). As well, A was significantly negatively affected by C_0 in Water F (Table 3-11).

Figure 3-11 Variation in inoculum concentration, C_0, among water samples. The one sample in Water D that had a higher C_0 than the others may have caused the significant difference in survival curve parameter values among water labels.

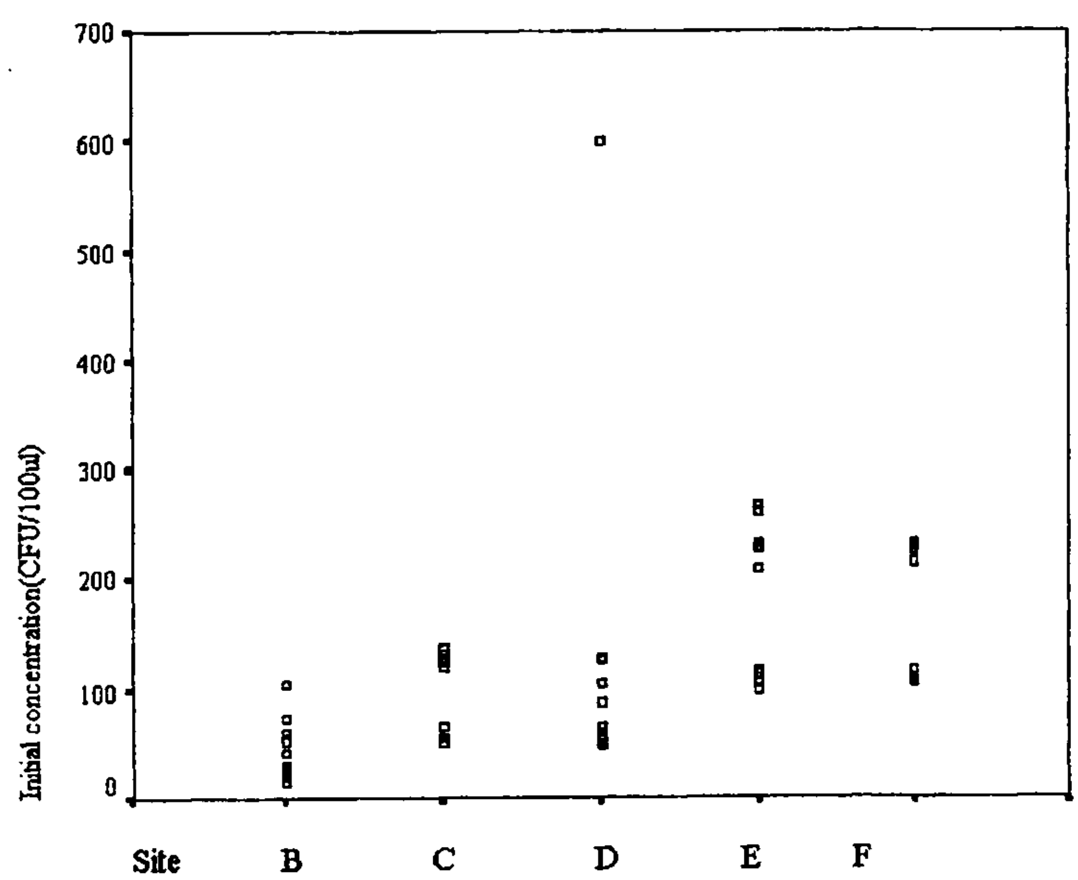
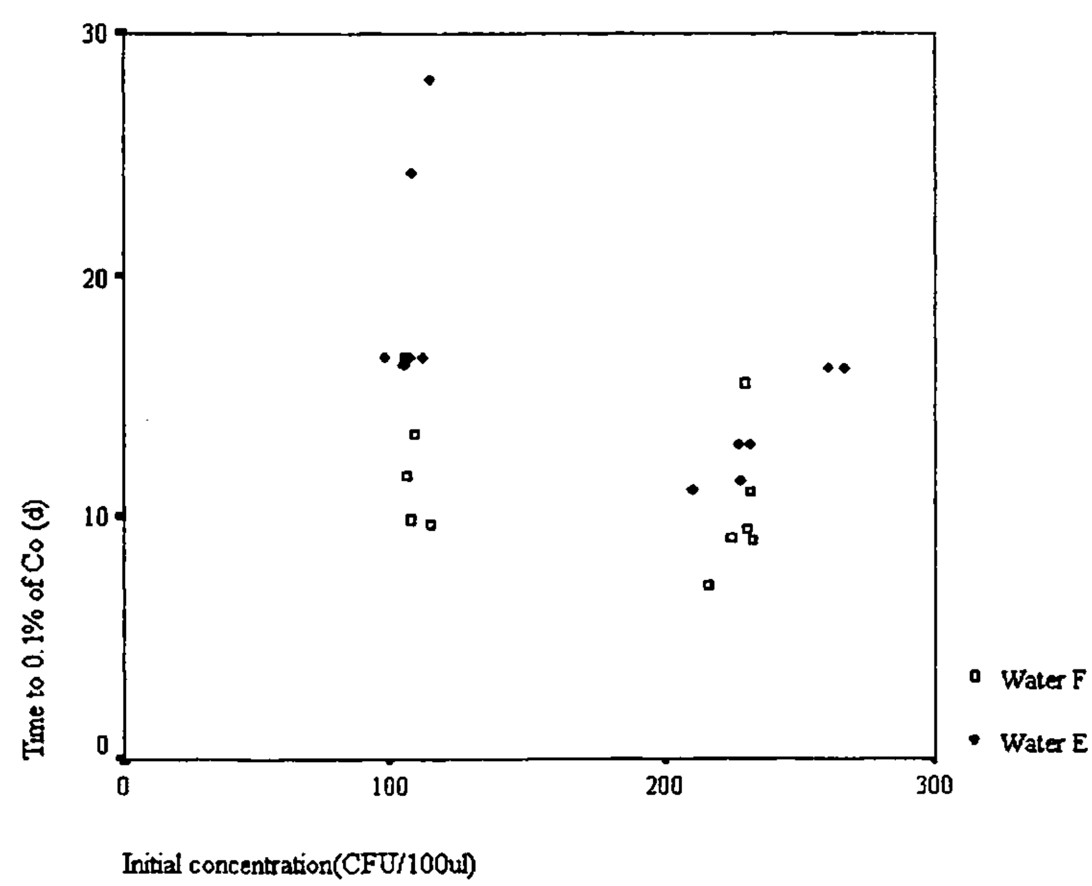


Table 3-11 Matrix of Spearman correlation coefficients, ρ, between C_0 and survival curve parameters for *E. coli* O157:H7 and *Salmonella* in each water sample.

		Water				
Parameter	Statistic	B	C	D	E	F
$t_{0.1}$	ρ	-0.399	-0.158	0.049	-0.608	-0.606
		(0.199)	(0.625)	(0.880)	(0.036)	(0.037)
Δt_{tail}	ρ	-0.203	0.004	0.231	-0.126	-0.483
		(0.527)	(0.991)	(0.471)	(0.697)	(0.111)
$\Delta t_{decline}$	ρ	-0.070	-0.207	-0.525	-0.420	-0.053
		(0.829)	(0.519)	(0.430)	(0.175)	(0.871)
A	ρ	-0.283	0.552	0.133	-0.252	-0.634
		(0.460)	(0.062)	(0.681)	(0.430)	(0.027)

Water samples were analysed separately since two-way ANOVA results indicated that the survival curve parameters varied among water samples and thus samples could not be pooled. *P* in parentheses.

Figure 3-12 Decrease in $t_{0.1}$ with increase in C_0 for *E. coli* O157:H7 and *Salmonella* in Waters E and F. Significant negative relationships were present for samples in each water sample ($P < 0.05$).



3.3.10 Shoulder Phase Analysis

The survival curves of the pathogens were either sigmoidal (with shoulder and tail phases) or biphasic (with tail but no shoulder phase)(Appendices 5 through 48). Pathogens in Waters A and B in filtered water were two times more likely to exhibit a shoulder phase response than in non-filtered, and they were also 1.77 times more common for *S. typhimurium* than for *E. coli* O157:H7 (Table 3-12). The presence of a shoulder phase was not associated with C_0 among samples in Waters B through F, (logistic regression model coefficient = -0.002, Wald P = 0.753).

Table 3-12 Number of samples with shoulder phase present or absent based on filtration and species, Waters A and B.

Factor	Condition	Presence	Absence
Filtration [a]	Filtered	24	20
	Non-filtered	12	32
Species [b]	*E. coli* O157:H7	13	31
	Salmonella	23	21

[a] Pearson $\chi^2 = 6.769$, $P = 0.009$, relative risk = 2.00

[b] Pearson $\chi^2 = 4.701$, $P = 0.030$, relative risk = 1.77

3.4 Discussion

It appears that the effects that environmental conditions temperature, autochthonous bacteria, and turbidity had on survival of *E. coli* O157:H7 and *Salmonella* in surface water were not strictly independent; interactions between factors may have influenced survival curve parameters. These pathogens were able to survive significantly longer during the decline phase at 10 °C than at 20 °C in non-filtered water (Table 3-10, Figure 3-10), similar to the trends shown by Flint (1987), Wang and Doyle, (1998) and Pokorny, (1988). However, this trend was not apparent in filtered water (Figure 3-11), suggesting filtration removed factors that may have influenced the duration of survival during the decline phase. This agrees with the results of studies by Wang and Doyle (1998) in which it was observed that the effect of temperature on pathogen survival was greater in lake water than in filtered water. Interactive effects of factors such as temperature and pH are recognised in food microbiology (Boddy and Wimpenny, 1992; Semancheck *et al.*, 1998; McDonald and Sun, 1999). In surface water, it may be possible that temperature has an interactive effect with micro-organisms indigenous to surface water on the survival of pathogens such as *E. coli* O157:H7 and *Salmonella*. The antagonistic effect that indigenous micro-organisms have on these pathogens may increase or be greatest at temperatures around 20 °C.

Removal of autochthonous bacteria did not appear to be the only cause of increased duration of decline phase in filtered water, since pathogen survival time in water from site A, which had no culturable autochthonous bacteria, increased with filtration (Figure 3-7). Although some bacteriophage are small enough to pass through the filter used (0.2μm pore size), others are larger (Tikhonenko, 1970) and may have been removed with filtration. The removal of phage or protozoa may have allowed the pathogens to survive longer in filtered Water A. In Water B increased pathogen survival with filtration may have been due to removal of indigenous phage, protozoa, and/or bacteria.

Since the duration of the decline phase of *E. coli* O157:H7 and *Salmonella* in surface water was greater at 10 °C than at 20 °C, one might assume the likelihood of these pathogens being isolated from environmental surface water samples would be greater at 10 °C than at 20 °C. However, it has been observed that prevalence of *Salmonella* in freshwater is greater during summer months than at other times of the year (Sharma and Rajput, 1996). This implies more faecal inputs or survival in protozoan biofilms.

Survival of *E. coli* O157:H7 and *Salmonella* may have been less at 20 °C than at 10 °C due to increased biofilm formation on the surface of sample bottle walls at the higher temperature. Pederson (1982) observed that *E. coli* attachment to glass surfaces increased with temperature. If cells became incorporated into biofilm, these cells would no longer be planktonic and the concentration of the pathogen in the water would have been reduced. However, if biofilms had developed in the sample bottle one might expect to observe prolonged survival due to the production of daughter cells from biofilms (Gilbert *et al*, 1993). However it seems unlikely that biofilm formation played a significant in this study as plate counts of samples following inversion and rapid swirling of sample bottles were not significantly different from those taken from bottles sampled directly (data not shown).

Filtration appears to have allowed a larger range of $\Delta t_{decline}$ (Figure 3-7), which may suggest that whatever was removed had a considerable limiting effect on pathogen survival during the decline phase. Prolonged pathogen survival in filtered water suggests that caution is advised in protection, treatment, and monitoring of filtered water distribution systems. If remediative measures are not complete, pathogen-contaminated water will persist for long periods of time and these organisms then may disperse over a large area.

While most survival models assume that the decline phase is linear (Whiting, 1993; Xiong *et al.*, 1999; Baranyi and Roberts, 1994), the present work approximated the non-linear decline phase as second-degree polynomial equations, and the acceleration parameter, *A* was developed as a measure of curvature of the decline phase. Positive curvature suggested a greater resistance to inactivation during the decline phase than a negative curvature. The decline of *E. coli* O157:H7 was, on average, convex, i.e. the mean value of acceleration was positive, while that of *S. typhimurium* was concave and the difference between the curvatures was significant (Section 3.3.7, Tables 3-6, 3-7), which suggests that *E. coli* O157:H7 was more resistant to inactivation during this phase. Jimenez and colleagues (1989) observed the rate of cell division was greater for *E. coli* than for *S. typhimurium* in the initial stages of survival in freshwater, but that this difference did not exist approximately 2 days later.

The ability of pathogens to survive stress is often due to phenotypic adaptation, rather than genotypic changes (Jordan, *et al.*, 1999; Abee and Wouters, 1999). Stress responses are induced when a challenge is present and can be shut off when the stress is removed (Fischer, *et al.*, 1998; Bloomfield *et al.*, 1998). All isolates in this study were cultured in nutrient broth at 37 °C overnight, thus, adaptations induced in waterborne isolates to respond to stresses in surface water were no longer required. However, in this study, strains isolated from sources other than water survived significantly longer during the tail phase than did those isolated from water, but only in filtered Water A and only when the samples were held at 10 °C. Reasons for this are unclear.

The difference in time parameters among the water samples may seem to be related to the difference in C_0. While it seems logical that a higher C_0 would result in a longer time of survival, there was a significant negative relationship between C_0 and $t_{0.1}$ in Waters E and F (Table 3-11, Figure 3-12). Possible reasons for this phenomenon are unclear.

When concentrations of bacteria are sufficiently high, autoinducer signal molecules may reach a critical threshold in a bacterial environment that may activate or repress target genes associated with the stress response (de Kievitt and Iglewski, 2000). These quorum-sensing systems are linked to the formation of clumps in *Yersinia pseudotuberculosis* (Atkinson, *et al.*, 1999). Clumping has been proposed as the mechanism behind the almost negligible decrease seen in CFUs during the shoulder phase. While cells on the surface of clumps inactivate, the cells within may remain viable and thus the clump of cells forms one CFU (Adams and Moss, 1995). Yet, in this experiment, higher inoculum concentration did not result in a greater occurrence of shoulder phases in the survival curves. Survival curves of pathogens in filtered water were more likely to have a shoulder phase than were those in non-filtered water (Table 3-12).

From the perspective of those charged with ensuring the safety of drinking water, the tail phase is as important as the decline phase in survival predictions of pathogens such as *E. coli* O157:H7. During the tail phase, pathogens may not be detectable as they are low in concentration. The tail phase was longer for the bovine isolate of *E. coli* O157:H7 and the laboratory strain of *S. typhimurium* at 10^0C than at 20 ^{0}C in filtered water. If filtration was used to treat a drinking water system and chlorine treatment was not used and *E. coli* O157:H7 of a strain similar to the bovine isolate used were present in the system, it appears that lower temperatures would prolong its ability to persist. It has been suggested that those cells that survive past the decline phase into the tail phase are a more resistant sub-population of the bacterial population (Cerf, 1977; Whiting, 1993).

Turbidity measures particulates in surface water that may include organic matter and bacterial nutrients (Loehr, 1984), and hence, surface water with high turbidity may increase the duration of bacterial survival. In this study turbidity was a factor in $\Delta t_{decline}$ in water held at 20 ^{0}C, but it appeared to have a negative influence on survival (Figure 3-6), i.e. some factor associated with the particulate matter in the water samples had a inhibitory effect on pathogen survival during the decline phase.

3.5 Conclusion

In this study, temperature, filtration, turbidity, and initial inoculum concentration affected the survival curve components of *E. coli* O157:H7 and *S. typhimurium* in surface water. Initial concentration was negatively related to $t_{0.1}$ in 2 water samples. All time measurement parameters were longer for pathogens in filtered water than in non-filtered water, but this was not due only to removal of autochthonous bacteria in filtered water. Higher temperature may have caused more rapid declines of populations of both pathogens in unfiltered water. Waterborne isolates did not have an advantage over isolates from other sources in surviving in surface water. The decline phase of *S. typhimurium* was generally concave, while that of *E. coli* O157:H7 was linear.

4 Discussion of Thesis

According to the results of the prevalence study presented here there is a risk to human health associated with the surface water in southern Alberta. The magnitude of risk and whether or not the level of risk is acceptable is not within the scope of this report. However, the fact that in many samples *Salmonella* serovars were isolated that have been associated with human disease outbreaks and *E. coli* O157:H7 was detected indicates that the surface water in the region is unsafe to drink if untreated. Caution must also be used with recreational activities and irrigation may contaminate animal feed and vegetable crops.

By strict definition, the title of this study is a misnomer. This was not a prevalence study of *E.coli* O157:H7 and *Salmonella* in surface water southern Alberta, as the sampling sites were not randomly selected throughout the region. Rather, it was a study of the prevalence of these pathogens in water from the selected sampling sites. Sites were chosen specifically to reflect upstream areas of interest, and to characterise contamination from a suspected point or non-point source.

Storm drain water samples were collected from the Lethbridge storm drain system, which collected the runoff from roofs, grassed areas and pavement. Sewage treatment plant samples were infrequent but useful in indicating quality of water that is discharged into the surface water system following treatment. The high pathogen prevalence in the storm drain and sewage treatment plant samples was greater than that of the study as a whole and suggests that when these are discharged into the surface water network, they are contributing to the pathogen load in surface water. Even though it could be argued that a high concentration of pathogens in the discharge would be dispersed with flowing water, they still may be a substantial contributor of pathogens into the water system. Waterborne transport, combined with prolonged survival and entrainment of pathogens on surfaces under water may increase the number of locations at which they can be detected. Formation of biofilms on underwater surfaces may serve as a long-term source of dispersal of pathogenic bacteria as daughter cells are released from the biofilm (Gilbert *et al.*, 1993). The results of the survival study presented here and those of other researchers (Rhodes and Kator, 1988; Jimenez *et al*, 1989; Wang and Doyle, 1998; Pokorny, 1988; Flint, 1987) indicates that, depending on the flow rate of the receiving water, the discharged pathogens could be detected over a longer distance downstream, and may have a far-reaching zone of potential for infection.

What is also evident from this survival study is that survival of pathogens in water may be influenced by one or more factors simultaneously, thus, it is important that effects of individual factors are teased out so that a more complete understanding of a given situation is achieved and more accurate predictions can be made. The combined effect of multiple factors may have a greater influence on the survival of pathogenic bacteria in surface water than do single factors, as witnessed in the interactive effect of temperature and indigenous bacteria on the duration of the decline phase of pathogens in Water B. It is acknowledged in food microbiology that effectiveness of microbial control agents increases when used in combination with complementary treatments (Boddy and Wimpenny, 1992).

The temperatures of $10^{\circ}C$ and $20^{\circ}C$ were selected for this study to represent upper and lower water temperatures that exist in a southern Alberta irrigation water distribution system. Simulating temperatures of irrigation systems was considered to be important as they supply water that may be used for residential consumption and domestic produce irrigation, and are, therefore, potential sources of human infection. Water flow in an irrigation distribution system fluctuates more rapidly than in natural water systems as flow is controlled to maintain adequate supplies to irrigation water users with a minimal of unused water. During low flow periods heat transfer from solar radiation through the depth of the water column is more rapid, and low velocity results in lower convective heat loss to the air. Duration of decline phase, $\Delta t_{decline}$ of *E. coli* O157:H7 and *Salmonella* was shown to decrease with increase in temperature in Waters B through F (Table 3-10). Consequently, low flow conditions, with warmer temperatures may cause pathogens to decrease more rapidly shortly after they enter the water system.

The fact that pathogen prevalence was higher in summer months than in the spring and fall, while the results of this experiment and others show pathogen survival is shorter at temperatures in the range of 20^0C than at 10^0C, may suggest that survival is not as important as other factors, such as increased shedding of pathogens from animal reservoirs or increase in contamination during summer months, in prevalence in surface water.

Filtration without chlorination would likely contribute to the ability of *E. coli* O157:H7 and *Salmonella* to survive over long periods of time and should, therefore, be advised against for the treatment of drinking water that may subsequently be contaminated with faecal material.

The use of indicator organisms to detect faecal contamination of water is useful in that respect. The suitability of these indicators to accurately describe the risk to human health due to the presence of enteric pathogens in the water might be questioned, as the correlation between pathogen presence and levels of indicator organisms was not consistent in this, and in other studies (Polo *et al.*, 1998; Arvanitidou *et al.*, 1997; Townsend, 1992; Luppi, *et al.* 1988; Efstratiou *et al.*, 1998). While there is a need for models to predict the presence of pathogenic bacteria based on indicator organism concentration (UNEP/WHO, 1996), one model satisfactory to all has not yet been developed (Efstratiou *et al.*, 1998).

5 Thesis Conclusions

The survey presented for *E. coli* O157:H7 prevalence in surface water presented is one of the largest performed to date. This pathogen was detected in 0.86% (13 of 1520) of the samples tested over a two-year period. *Salmonella* species were isolated in 6.04% (88 of 1458) of the samples. While there was no significant change between the years in the prevalence in *Salmonella,* there was an increase in the prevalence in *E. coli* O157:H7 from 1999 to 2000. Peaks in prevalence of both species occurred from the middle of July to eary August. The presence of *E. coli* O157:H7 and *Salmonella* serotypes associated with outbreaks of disease in humans suggests some risk to human health related to consumption of water from which these pathogens were isolated.

While prevalence of pathogens in surface water is a factor in the risk of infection associated with use of surface water, their survival also plays a role. Models of pathogen survival provide estimates of concentration as it changes with time and as it varies with survival conditions. Survival curves consist of 3 components, shoulder, decline, and tail phases. In this study, autochthonous bacteria, temperature, filtration, turbidity, and initial inoculum concentration affected the survival curve components of *E. coli* O157:H7 and *S. typhimurium* in surface water. Initial concentration was negatively related to $t_{0.1}$, but only in 2 water samples. All time measurement parameters were longer for pathogens in filtered water than in non-filtered water, but this was not due only to removal of autochthonous bacteria in filtered water. Higher temperature resulted in more rapid decline in pathogen in unfiltered water. Waterborne isolates did not have an advantage in terms of survival over isolates from other sources.

6 References

Abee, T. and J.A. Wouters. 1999. Microbial stress response in minimal processing. *Int. J. Food. Microbiol.* 50:65-91.

Adams, M.R. and M.O. Moss. 1995. Food microbiology. The Royal Society of Chemistry, Cambridge.

Adensiyun, A.A. 1999. Absence of *Escherichia coli* O157 in a survey of wildlife from Trinidad and Tobago. *J. Wildl. Dis.* 35:115-20.

Afrasiabi SN and N.S. Waleh. 1986. Production of colicin E5 by epidemic strains of *Salmonella typhimurium* var. Copenhagen. *Microbios.* 45:209-12

Agriculture and Agri-food Canada Research Branch. 2000. Meteorological Data at the Agricultural Research Station. http://res2.agr.ca/lethbridge/weather/y1999_l.txt Accessed 2001, Feb. 22.

Alberta Agriculture. 1991. The location of cattle production in Alberta. Alberta Agriculture, Edmonton.

Alberta Agriculture, Food and Rural Development. 2000. Crops grown in the thirteen irrigation districts. http://www.agric.gov.ab.ca/irrigate/stats/index.html Accessed 2001, Feb. 21.

Akashi, S., K. Joh, A. Tsuji, H. Ito, H. Hoshi, T. Hayakawa, J. Ihara, T. Abe, M. Hatori, T. Mori, T. Nakamura. 1994. A severe outbreak of haemorrahagic and haemolytic uraemic syndrome associated with *Escherichia coli* O157:H7 in Japan. *Eur. J. Pediatr.* 153:650-5.

American Public Health Association. 1985. Standard Methods for the Examination of Water and Wastewater. American Public Health Association , Washington, D.C..

Andrews, W.H., V.R. Bruce, G. June, F. Sathcell, P. Sherrod. 1992. *Salmonella. In* Food and Drug Administration Bacteriological Analytical Manual, 7th edition. AOAC International, Arlington.

Anonymous. 1992. Hospital outbreak of *Salmonella enteriditis* infection--Ontario. *Can Commun. Dis. Rep.* 18:57-60

Anonymous. 1999. Outbreak of *Escherichia coli* O157:H7 and *Campylobacter* among attendees of the Washington County Fair-New York, 1999. *MMWR* 48:803-5.

Armstrong, G.L., J. Hollingsworth, J.G. Morris. 1996. Emerging foodborne pathogens: *Escherichia coli* O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. *Epidemiol. Rev.* 18:29-51.

Arvaitidou, M., A. Papa, T.C. Constantinidis, V. Danielides, V. Katsouyannopoulos. 1997. The occurrence of *Listeria* spp. and *Salmonella* spp. in surface waters. *Microbiol. Res.*152:395-97.

Atkinson, S., J. P. Throup, G,S, Stewart, P. Williams. 1999. A hierarchical quorum-sensing system in *Yersinia pseudotuberculosis* is involved in the regulation of motility and clumping. *Mol. Microbiol.* 33:1267-77.

Atlas, R.M. 1997. Principles of Microbiology. Wm. C. Brown Publishers, Dubuque.

Ausubel, F.M., R. Brent, R.E. Kingston, D.D Moore, J.G. Seidman, J.A. Smith, K. Struhl. 1995. Short protocols in molecular biology (3rd edition). John Wiley and Sons, New York.

Bach KK, C. Cupp, D. Brewster, D. Wester, M. Keefe. 1999. Effects of activated macrophages and fibroblast growth factor on random skin flap survival in swine. *Laryngoscope*. 109:1156-9.

Baird-Parker, A.C. 1990. Food borne salmonellosis. *Lancet.* 336:1231-5.

Bahlaoui, M.A., B. Baleux, M.A. Frouji. 1998. The effect of environmental factors on bacterial populations and community dynamics in high rate oxidation ponds. *Water Environ. Res.* 70:1186-97.

Baranyi, J. and T.A. Roberts. 1994. A dynamic approach to predicting bacterial growth in food. *Int. J. Food. Microbiol.* 23:277-94.

Barrell, R.A.E., P.R. Hunter, G. Nichols. 2000. Microbiological standards for water and their relationship to human health. *Commun. Dis. Public Health.* 3:8-13.

Barwick, R.S., D.A. Levy, G.F. Craun, M.J. Beach, R.L. Calderon. 2000. Surveillance for waterborne-disease outbreaks-United States, 1997-1998. *MMWR.* 49(SS4)1-35.

Baxter DN, S. Morton. 1987. An outbreak of *S. indiana* in a maternity unit: implications for control policies. *Community Med.* 9: 365-71

Bean, N.H., J.S. Golding, C.Lao, F.J.Angulo. 1996. Surveillance for foodborne-disease outbreaks-United States, 1988-1992. *MMWR* 45(SS-5):1-73.

Blaser, M.J., L.S. Newman. 1982. A review of human salmonellosis: I. Infective dose. *Rev. Infect. Dis.* 4:1096-106.

Bloomfield, S.F., G. Stewart, C.E.R. Dodd, I.R. Booth, E.G.M Power. 1998. The viable but non-culturable phenomenon explained? *Microbiol.* 144:1-2.

Boddy, L. and J.W.T. Wimpenny. 1992. Ecological concepts in food microbiology. *J. Appl. Bacteriol.* Supplement 73:23S-38S.

Boyce, T.G , D.L. Swerdlow and P.M. Griffin. 1995. *Escherichia coli* O157:H7 and the hemolytic-uremic syndrome. *N. Engl. J. Med.* 333:364-68.

Buchanan, R.L., R.C. Whiting. 1996. Risk assessment and predictive microbiology. *J. Food Prot.* S31-6:31-6.

Buchanan, R.L., M.H. Golden, R.C.Whiting. 1993. Differentiation of the effects of pH and lactic or acetic acid concentration on the kinetics of *Listeria monocytogenes* survival. *J. Food Prot.* 56:474-8.

Byrd, J.J., H.S.Xu,.R.R. Colwell. 1991. Viable but nonculturable bacteria in drinking water. *Appl. Environ. Microbiol.* 57:875-8.

Calleja, G.B. 1984. Microbial aggregation. CRC Press, Boca Raton.

Canadian Council of Ministers of the Environment. 1999. Canadian Water Quality Guidelines for the Protection of Agricultural Water Uses.

Carter, G.R. 1979. Diagnostic procedures in veterinary bacteriology and mycology. Charles C. Thomas, Springfield.

Cerf, O. 1977. A review: Tailing of survival curves of bacterial spores. *J. Appl. Microbiol.* 42:1-19.

Chang, Y.H. 2000. Prevalence of *Salmonella* spp. in poultry broilers and shell eggs in Korea. *J. Food Prot.* 63:655-8.

Chao, W.L., R.S. Chen. C.L. Tai. 1988. Factors affecting the survival of pathogenic bacteria in subtropical river water. *Chinese J. Microbiol. Immunol.* 21:85-92.

Chapman, P.A., C.A. Siddons, A.T. Cerdan Malo, M.A. Harkin. 1997. A 1-year study of *Escherichia coli* O157 in cattle, sheep, pigs and poultry. *Epidemiol. Infect.* 119:245-50.

Chapman, P.A., D.J. Wright, C.A. Siddons. 1994. A comparison of immunomagnetic separation and direct culture for the isolation of verocytotoxin-producing *Escherichia coli* O157 from bovine faeces. *J. Med. Microbiol.* 40:424-7.

Chiu, C.H., J.T. Ou. 1996. Rapid identification of *Salmonella* serovars in feces by specific detection of virulence genes, *invA* and *spvC*, by an enrichment broth culture-multiplex PCR combination assay. *J. Clin. Microbiol.* 34:2619-22.

Cizek A, I. Literak, K. Hejlicek, F.Treml, J. Smola. 1994. *Salmonella* contamination of the environment and its incidence in wild birds. *Zentralbl Veterinarmed [B].* 41:320-7.

Cornick, N.A., S.L. Booher, T.A. Casey, H.W. Moon. 2000. Persistent colonization of sheep by *Escherichia coli* O157:H7 and other *E. coli* pathotypes. *Appl. Environ. Microbiol.* 66:4926-34.

D, Aoust, J.Y. 1997. *Salmonella* species. p.129-205. *In*: Doyle, M.P., L.R. Beuchat, T.J. Montville (eds) Food microbiology: Fundamentals and frontiers. ASM Press, Washington, D.C.

D' Aoust, J.Y. and U. Purvis. 1998. Isolation and identification of *Salmonella* from foods (Health Protection Branch Method MFHPB-20). Health Protection Branch. Ottawa.

DeSmedt,J.M, .R.F Bolderdijk, H.F. Rappold, D.Lautenschlager. 1986. Rapid *Salmonella* detection in foods by motility enrichment on a modified semi-solid Rappaport Vassiliadis medium *J.Food Prot.* 49:510-4

DeSmedt,J.M., R.F Bolderdijk. 1987. Dynamics of *Salmonella* isolation with modified semi-solid Rappaport Vassiliadis medium. *J. Food Prot.* 50: 658-61.

DeVinney R, A. Gauthier, A. Abe, B.B. Finlay. 1999. Enteropathogenic Escherichia coli: a pathogen that inserts its own receptor into host cells. *Cell Mol. Life Sci.* 55:961-76.

Dodd, C.E.R, R.L. Sharman, S.F. Bloomfield, I.R. Booth, G.S. Stewart. 1997. Inimical processes: Bacterial self-destruction and sub-lethal injury. *Trends Food Sci. Tech.* 8:238-41.

Donnenberg, M.S. and J.B.Kaper. 1992. Enteropathogenic *Escherichia coli Infect. Immun.* 60:3953-61.

Doyle, M.P., T.Zhao, J. Meng, S. Zhao. 1997. *Escherichia coli* O157:H7. p.171-91. *In:* Doyle, M.P., L.R. Beuchat, T.J. Montville (eds) Food microbiology: Fundamentals and frontiers. ASM Press, Washington, D.C.

Edel, W., M. Van Schothorst, E.H. Kampelmacher. 1976. Epidemiological studies on Salmonella in a certain area ("Walcheren Project") I. The presence of Salmonella in man, pigs, insects, seagulls, and in foods and effluents. *Zbl. Bakt. Hyg., I. Abt. Orig.* 325:476-84.

Efstratiou, M.A., A. Mavridou, S.C. Richardson, J.A. Papadakis. 1998. Correlation between indicator organisms with *Salmonella* spp., *Staphylococcus aureus* and *Candida albicans* in sea water. *Lett. Appl. Microbiol.* 26:342-346.

Ewald, P.W., J.B. Sussman,M.T. Distler, C. Libel, W. P. Chammas, V.J. Dirita, C.A. Salles, A.C. Vicnete, I. Heitmann, F. Cabello. 1998. Evolutionary control of infectious disease: Prospects for vectorborne and waterborne pathogens. *Mem. Inst. Oswaldo Cruz* 93:567-76.

Faustini A, Sangalli M, Fantasia M, Manganello R, Mattaccini E, Trippanera R, Spera D, La Rosa U, Topi MT, Forastiere F, Perucci CA. 1998. An outbreak of *Salmonella hadar* associated with food consumption at a building site canteen. *Eur. J. Epidemiol.* 14:99-106

Federal-Provincial Subcommittee on Recreational Water Quality, 1992. Guidelines for Canadian Recreational Water Quality. Health Canada, Ottawa.

Federal-Provincial Subcommittee on Drinking Water, 1999. Summary of Guidelines for Canadian Drinking Water Quality. Health Canada, Ottawa.

Finlay, B.B. 1994. Molecular and cellular mechanisms of *Salmonella* pathogenesis. *Curr Mirobiol. Immunol.* 192:163-85.

Fischer, D., A.Teich, P. Neubauer, R.Hengge-Aronis. 1998. The general stress sigma factor σ^S of *Escherichia coli* is induced during diauxic shift from glucose to lactose. *J. Bacteriol.* 180:6203-6.

Flint, K.P. 1987. The long-term survival of *Escherichia coli* in river water. *J. Appl. Bacteriol.* 63:261-70.

Galland JC, J.K. House, D.R. Hyatt, L.L. Hawkins, N.V. Anderson, C.K. Irwin, B.P. Smith. 2000. Prevalence of *Salmonella* in beef feeder steers as determined by bacterial culture and ELISA serology. *Vet. Microbiol.* 6:143-51

Gannon, V.P.J., S. D'Souza, T. A. Graham, R.K. King, K. Rahn and S. Read. 1997. Use of the flagellar H7 gene as a target in multiplex PCR assays and improved specificity in identification of enterohaemorrhagic *Escherichia coli* strains. *J. Clin. Microbiol.* 35:656-662.

Gérard, F., A.Dri, P.L. Moreau. 1999. Role of *Escherichia coli* RpoS, LexA and H-NS global regulators in metabolism and survival under aerobic, phosphate-starvation conditions. *Microbiol.* 145:1547-62.

Gerstman, B.B. 1998. Epidemiology kept simple: An introduction to class and modern epidemiology. Wiley-Liss, Inc., New York.

Gilbert, P., D.J. Evans, M.R.W. Brown. 1993. Formation and dispersal of bacterial biofilms *in vivo* and *in situ*. *J. Appl. Bacteriol.* S74:67-78.

Griffin, P.M. and R.V. Tauxe. 1991. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemmorhagic *E. coli*, and the associated hemolytic uremic syndrome. *Epidemiol. Rev.* 13:60-98.

Griffin, P.M. 1995. *Escherichia coli* O157:H7 and other enterohaemorrhagic *Escherichia coli*. p.739-61. *In*: Blaser, M.J., P.D. Smith, J.I.Radvin, H.B. Greenberg, R.L. Guerrant. (ed) Infections of the gastrointestinal tract. New York, Raven Press.

Griffiths, M.W. 1994. Predictive modelling: applications in the dairy industry. *Int. J. Food. Microbiol.*23: 305-15.

Hancock DD, TE Besser, DH Rice, DE Herriott, PI Tarr. 1997. A longitudinal study of *Escherichia coli* O157 in fourteen cattle herds. *Epidemiol Infect* 118:193-5

Hengge-Aronis, R. 1993. Survival of hunger and stress: the role of *rpoS* in early stationary phase gene regulation in *E. coli*. *Cell.* 72:165-8.

Higgins, R., R. Malo, E. René-Roberge, R.Gauthier. 1982. Studies on the dissemination of *Salmonella* in nine broiler-chicken flocks. *Avian Dis.* 26:26-32.

Hobbie, J.E., R.J.Daley, S. Jasper. 1977. Use of nucleopore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.* 33:1225-8.

Hohls, D., G.duPlessis, S.N. Venter, M.C. Stynberg, C.M. De Wet, N. Rodda, R. Kfir. 1995. Estimation of the fate of microbial water quality contaminants in a South African river. *Wat. Sci. Tech.* 31:271-4.

Holcomb, D.L., M.A. Smith, G.O.Ware, Y.C. Hung, R.E. Brackett, M. P. Doyle. 1999. Comparison of six dose-response models for use with food-borne pathogens. *Risk Anal.* 19:1091-1100.

Hosmer, D.W., S. Lemeshow. 1989. Applied logistic regression. John Wiley and Sons, New York.

Hull, S. 1997. *Escherichia coli* lipopolysaccharide in pathogenesis and virulence. p. 145-67. *In* Sussman, M.(ed) *Escherichia coli*: Mechanisms of virulence. Cambridge University Press, Cambridge.

Iversen, G.R. and H. Norpoth. 1987. Analysis of variance. SAGE Publications, Newbury Park.

Jansson, J.K. and J.I. Prosser. 1997. Quantification of the presence and activity of specific microorgnisms in nature. *Mol. Biotechnol.* 7:3-20.

Jimenez, L., I. Muniz, G.A. Toranzos, T.C. Hazen. 1989. Survival and activity of *Salmonella typhimurium* and *Escherichia coli* in tropical freshwater. *J. Appl. Bacteriol.* 67:61-9.

Jones, I.G., M. Roworth. 1996. An outbreak of *Escherichia coli* O157 and Campylobacteriosis associated with contamination of a drinking water supply. *Pub. Health.* 110:277-82.

Jordan, K.N., L.Oxford, C.P. O'Byrne. 1999. Survival of low-pH stress by *Escherichia coli* O157:H7: Correlation between alterations in the cell envelope and increased acid tolerance. *Appl. Environ. Microbiol.* 65:3048-55.

Kaplan, C. 1997. *Infection and environment.* Butterworth-Heinemann. Oxford.

Keene, W.E., J.M. McAnulty, F.C. Hoesly, L.P. Williams, K.Hedberg, G.L. Oxman, T.J. Barrett, M.A. Pfaller, D.W. Fleming. 1994. A swimming-associated outbreak of hemorrhagic colitis caused by *Escherichia coli* O157:H7 and *Shigella sonnei. N. Engl. J. Med.* 331:579-84.

Kievit, T.R. and B.H. Iglewski. 2000. Bacterial quorum sensing in pathogenic relationships. *Infect. Immun.* 68:4839-49.

King, C.H., E.B. Shotts, R.E. Wooley, K.G. Porter. 1988. Survival of coliforms and bacterial pathogens within protozoa during chlorination. *Appl. Environ. Microbiol.* 54:3023-33.

Kondro, W. 2000. *E. coli* outbreak deaths spark judicial inquiry in Canada. *Lancet.* 355: 2058.

Konowalchuk, J., J.I.Spiers, and S. Stavric. 1977. Response to a cytotoxin of *Escherichia coli. Infect. Immun.* 18:775-9.

Koplan, J.P., R.D. Deen. W.H. Swanston, B. Tota. 1978. Contaminated roof-collected rainwater as a possible cause of an outbreak of salmonellosis. *J. Hyg. Camb.* 81:303-9.

Kramer, M.H., B.L. Herwaldt, G.F. Craun, R.L. Calderon, D.D. Juranek. 1996. Surveillance from waterborne-disease outbreaks-United States, 1993-1994. *MMWR.* 45:1-33.

Kurath, G. and R.Y. Morita. 1983. Starvation-survival physiological studies of a marine *Pseudomonas* sp. *Appl. Environ. Microbiol.* 45: 1206-11.

Lammerding, A.M. and A. Fazil. 2000. Hazard identification and exposure assessment for microbial food safety risk assessment. *Int. J. Food Microbiol.* 58:147-57.

LeChevalier, M.W, W. Schulz, R.G. Lee. 1991. Bacterial nutrients in drinking water. *Appl. Environ. Microbiol.* 57: 857-62.

Lee, I.S., J. Lin, H.K Hall, B. Bearson, J.W. Foster. 1995. The stationary-phase sigma factor σ^S (RpoS) is required for a sustained acid tolerance response in virulent *Salmonella typhimurium. Mol. Microbiol.* 17:155-67.

Lehmacher A, Bockemuhl J, Aleksic S. 1995. Nationwide outbreak of human salmonellosis in Germany due to contaminated paprika and paprika-powdered potato chips. *Epidemiol. Infect.* 115:501-11.

Le Minor, L. 1984. Salmonella. p.427. *In*: Krieg, N.R. (ed). Bergey's manual of systematic bacteriology, vol 1. Williams and Wilkins, Baltimore.

Lethbridge Northern Irrigation District. *Just add water.* (Brochure) Distributed Sept. 2000.

Levy B.S., W. McIntire, L. Damsky, R. Lashbrook, J. Hawk, G.S. Jacobsen, B. Newton. 1975. The Middleton outbreak: 125 cases of foodborne salmonellosis resulting from cross-contaminated food items served at a picnic and a smorgasbord. *Am. J. Epidemiol.* 101:502-11

Levy, D.A., M.S. Bens., G.F. Craun, R.L. Calderon, B.L. Herwaldt. 1998. Surveillance for waterborne-disease outbreaks-United States, 1995-1996. *MMWR.* 47(SS-5):1-34.

Li, Y., E. Frey, A.M.R. Mackenzie, B.B. Finlay. 2000. Human response to *Escherichia coli* O157:H7 infection: antibodies to secreted virulence factors. *Infect. Immun.* 68(9):5090-95.

Linton, R.H., W.H. Carter, M.D. Pierson, C.R. Hackney, J.D. Eifert. 1996. Use of a modified Gompertz equation to predict the effects of temperature, pH, and NaCl on the inactivation of *Listeria monocytogenes* Scott A heated in infant formula. *J. Food Prot.* 59:16-23.

Loehr, R.C. 1984. *Pollution control for agriculture.* Academic Press, Orlando.

Loewen, P.C., B.Hu, J Strutinsky, R Sparling. 1998 Regulation in the *rpoS* regulon of *Escherichia coli. Can. J. Microbiol.* 44:707-17.

Luppi, A, G. Bucci, P. Maini, J. Rocourt. 1988. Ecological survey of *Listeria* in the Ferrara area (Northern Italy). *Zbl. Bakt. Hyg. A.* 269:266-75.

Macler, B.A. and S. Regli. 1993. Use of microbial risk assessment in setting US drinking water standards. *Int. J. Food. Microbiol.* 18:245-56.

Marks, H.M. , Coleman, M.E., C.T. Jordan-Lin, T. Roberts. 1998. Topics in microbial risk assessment: Dynamic flow tree process. *Risk Anal.* 18:309-28.

McCambridge, J. and T.A. McMeekin. 1980. Relative effects of bacterial and protozoan predators on survival of *Escherichia coli* in estuarine water samples. *Appl. Environ. Microbiol.* 40:907-11.

McCambridge, J. and T.A. McMeekin. 1981. Effect of solar radiation and predacious micro-organisms on survival of fecal and other bacteria. *Appl. Environ. Microbiol.* 41:1083-7.

McDonald, K. and D.W. Sun. 1999. Predictive food microbiology for the meat industry: a review. *Int. J. Food. Microbiol.* 52:1-27.

McFeters, G.A. 1989. Detection and significance of injured indicator and pathogenic bacteria in water. p.179-210. *In*: Ray (ed.). Injured index and pathogenic bacteria: occurrence and detection in foods, water, and feed. p.179-210. CRC Press, Boca Raton.

McGowan, K.L., E. Wickersham, N.A. Strockbine. 1989. *Escherichia coli* O157:H7 from water. *Lancet* I:987-8.

McGuigan, K.G., T.M Joyce, R.M. Conroy. 1999, Solar disinfection: use of sunlight to decontaminate drinking water in developing countries. *J. Med. Microbiol.* 48:785-7.

McKay, A.M. 1992. Viable but non-culturable forms of potentially pathogenic bacteria in water. *Lett. Appl. Microbiol.* 14:129-35.

Michel, R., K.D. Muller, R. Amann., E.N. Schmid. 1998. *Legionella*-like rods multiplying within a strain of *Acanthamoeba* sp. isolated from drinking water. *Parasito. Res.* 84:84-8.

Moe, C. L. 1997. Waterborne transmission of infectious agents. p.136-52. *In* Hurst, C.J., G.R. Knudsen, M.J. McInerney, L.D. Stetzenbach, M.V. Walter (eds) Manual of environmental microbiology. ASM Press, Washington, D.C.

Muela, A., I. Arana, J.I. Justo, C. Seco, I. Barcina. 1999. Changes in DNA content and cellular death during a starvation-survival process of *Escherichia coli* in river water. *Microbiol. Ecol.* 37:62-9.

Nataro, J.P. and J.B. Kaper. 1998. Diarrhegenic *Escherichia coli*. *Clin. Microbiol. Rev.* 11:142-201.

Natural Resources Canada. 2001. The National Atlas of Canada-Facts About Canada-Rivers. http://www.atlas.gc.ca/english/facts/rivers.html Accessed 2001, Feb.21.

Natural Resources Service, Water Management Division, Alberta Environment. 1999. Surface Water Quality Guidelines for use in Alberta. Environmental Sciences Division, Edmonton.

Neumann, D.A., J.A. Foran. 1997. Assessing the risks associated with exposure to waterborne pathogens: An expert panel's report on risk assessment. *J. Food Prot.* 60:1426-31.

O'Brien, A.D., J.W. Newland, S.F. Miller, R.K. Holmes. 1984. Shiga-like toxin-converting phages from *Escherichia coli* strain that cause hemorrhagic colits or infantile diarrhea. *Science* 226:694-6.

Olsen, S.J., L.C. MacKinon, J.S. Goulding, N.H Bean. L. Slutsker. 2000. Surveillance for food borne-disease outbreaks-United States, 1993-1997. *MMWR*. 49 (SS1)1-51.

Olson, B.H. 1993. Pathogen occurrence in source waters: factors affecting survival and growth. p.83-97. *In:* Craun, G.F. (ed) Safety of water disinfect ion: balancing chemical and microbial risks. ILSI Press, Washington, D.C.

Oosterom, J., R. Dekker, G.J.A. de Wilde, F. van Kempen-de Troy, G.B. Engels. 1985. Prevalence of *Campylobacter jejuni* and *Salmonella* during pig slaughtering. *Vet. Q.* 7:31-4.

Parish ME. 1998. Coliforms, *Escherichia coli* and *Salmonella* serovars associated with a citrus-processing facility implicated in a salmonellosis outbreak. *J Foot Prot.* 61:280-4.

Pedersen, K. 1982. Factors regulating microbial biofilm development in a system with slowly flowing seawater. *Appl. Environ. Microbiol.*. 44:1196-1204.

Polo, F. , M.J. Figueras, I Inza, J. Sala, J.M. Fleisher, J. Guarro.1998. Relationship between presence of *Salmonella* and indicators of faecal pollution in aquatic habitats. *FEMS Microbiol. Lett.* 160:253-6.

Pokorny, J. 1988. Survival and virulence of Salmonellae in water. *J. Hyg. Epidemiol. Microbiol. Immunol.* 32:361-6.

Polotsky, Y.E., E. Dragunsky, T. Khavkin. 1994. Morphological evaluation of the pathogenisis of bacterial enteric infections. *Crit. Rev. Microbiol.* 20:161-208.

Pommepuy, M., M. Butin, A Derrien, M. Gourmelon, R.R. Colwell, M. Cormier. 1996. Retention of enteropathogenicity by viable but nonculturable *Escherichia coli* exposed to seawater and sunlight. *Appl. Environ. Microbiol.* 62:4621-6.

Postgate, J.R. 1976. Death in macrobes and microbes. p.1-18. *In*: Gray and Postgate (eds) The survival of vegetative microbes. Cambridge University Press, Cambridge.

Prasad, R., A.K. Chopra, J.W. Peterson, R. Pericas, C.W. Houston. 1990. Biological and immunological characterization of a cloned cholera toxin-like enterotoxin from *Salmonella typhimurium*. *Microb. Pathog.* 9:315-29.

Reeve C.A., P.S. Amy, A. Matin. 1984. Role of protein synthesis in the survival of carbon-starved *Escherichia coli* K-12. *J. Bacteriol.* 160:1041-6.

Rhodes, M.W. and H. Kator. 1988. Survival of *Escherichia coli* and *Salmonella* in estuarine environments. *Appl. Env. Microbiol.* 54:2902-7.

Rice, E.W. and C.H. Johnson. 2000. Short communication: Survival of *Escherichia coli* O157:H7 in dairy cattle drinking water. *J. Dairy Sci.* 83:2021-3.

Roszak, D.B., D.J.Grimes, R.R. Colwell. 1983. Viable but nonrecoverable stage of *Salmonella enteritidis* in aquatic systems. *Can. J. Microbiol.* 30:334-8.

Roszak, D.B. and R.R. Colwell. 1987. Survival strategies of bacteria in the natural environment. *Microbiol. Rev.* 51:365-79.

Rowe, P.C., E. Orrbine, G.A. Wells, P.N. McLaine *et al.* 1991. Epidemiology of hemolytic-uremic syndrome in Canadian children from 1086 to 1988. *J. Pediatr.* 119:218-24.

Ruiz J, Nunez ML, Diaz J, Sempere MA, Gomez J, Usera MAJ. 1996. Note: comparison of media for the isolation of lactose-positive *Salmonella. J. Appl. Bacteriol.* 81:571-4.

Rushdy AA, J.M. Stuart, L.R. Ward, J. Bruce, E.J. Threlfall, P. Punia, J.R. Bailey. 1998. National outbreak of *Salmonella senftenberg* associated with infant food. *Epidemiol Infect.* 120:125-8.

Sanderson, M.W., J.M. Gay, D.D. Hancock, C.C. Gay, L.K. Fox, T.E. Besser. 1995. Sensitivity of bacteriologic culture for detection of *Escherichia* coli O157:H7 in bovine feces. *J. Clin. Microbiol.* 33:2616-9.

Santo Domingo, J.W, S. Harmon, J. Bennett. 2000. Survival of *Salmonella* species in river water. *Curr. Microbiol.* 40:409-17.

Sargeant, J.M., J.R. Gillespie, R.D. Oberst, R.K. Phebus, D.R. Hyatt, L.K. Bohra, J.C. Galland. 2000. Results of a longitudinal study of the prevalence of *Escherichia coli* O157:H7 on cow-calf farms. *Am. J. Vet. Res.* 61:1375-9.

Scheil W, Cameron S, Dalton C, Murray C, Wilson D. 1998. A South Australian *Salmonella mbandaka* outbreak investigation using a database to select controls. *Aust. N. Z. J. Public Health.* 22:536-9

Scotland, S.M., H.R. Smith and B.Rowe. 1985. Two distinct toxins active on Vero cells from *Escherichia coli* O157. *Lancet.* 2:885-6.

Semanchek, J.J. and D.A. Golden. 1998. Influence of growth temperature on inactivation of *Escherichia coli* O157:H7 by heat, acid, and freezing. *J. Food Prot.* 61:395-401.

Shallow, S., M Samuel, A. McNees, G. Rothrock, D. Vugia, S. Waterman, T. Fiorentino, R. Marcus, G. Kazi, P. Mshar, *et al.* 1999. Incidence of foodborne illness: Preliminary data from the foodborne diseases active surveillance network. *MMWR.* 48:189-92.

Shapiro R, Ackers ML, Lance S, Rabbani M, Schaefer L, Daugherty J, Thelen C, Swerdlow D. 1999. *Salmonella Thompson* associated with improper handling of roast beef at a restaurant in Sioux Falls, South Dakota. *J. Food Prot.* 62:118-22.

Sharma, A. and S. Rajput. 1996. *Salmonella* as an index of pollution of fresh-water environments. *Environ. Monit. Assess.* 41:67-76.

Shere, J.A., K.J. Bartlett, C.W. Kaspar. 1998. Longitudinal study of *Escherichia coli* O157:H7 dissemination on four dairy farms in Wisconsin. *Appl. Environ. Microbiol.* 64:1390-9.

Sibille, I., T. Sime-Mgando, L. Mathieu, J.C. Block. 1998. Protozoan bacterivory and *Escherichia coli* survival in drinking water distribution systems. *Appl. Environ. Microbiol.* 64:197-202.

Stevenson, L.H. 1978. A case for bacterial dormancy in aquatic systems. *Microbiol. Ecol.* 4:127-33.

Stolley, P.D. and T. Lasky. 1998. Investigating disease patterns: the science of epidemiology. Scientific American Library, New York.

Su, C. 1996. 1996 Census of agriculture. http://www.agric.gov.ab.ca/economic/census/south/land.html Accessed 2001, Feb. 21.

Swerdlow, D.L., B.A. Woodruff, R.C. Brady, P.M Griffin, S. Tippen, D.Donnell, E. Geldreich, B.J. Payne, A. Meyer, J.G. Wells, K.D. Greene, M. Bright, N.H. Bean, P.A. Blake. 1992. A waterborne outbreak in Missouri of *Escherichia coli* O157:H7 associated with bloody diarrhea and death. *Ann Intern. Med.* 117: 812-818.

Synnott MB, M. Brindley, J. Gray, J.K. Dawson. 1998. An outbreak of *Salmonella agona* infection associated with precooked turkey meat. *Commun. Dis. Public Health.*1:176-9.

Tarr, P.I. 1994. *Escherichia coli* O157:H7: clinical, diagnostic, and epidemiological aspects of human infection. *Clin. Infect. Dis.* 20:1-10.

Tatsuno, I., H. Kimura, A.Okutani, K. Kanamaru, H. Abe, S. Nagai, K. Makino, H. Shinagawa, M. Yoshida, K. Sato, J. Nakamoto, T. Tobe, C. Sasakawa. 2000. Isolation and characterisation of Mini-Tn5Km2 insertion mutants of enterohemorrhagic *Escherichia coli* O157:H7 deficient in adherence to Caco-2 cells. *Infect. Immun.* 68:5943-52.

Tikhonenko, A.S. 1970. Ultrastructure of bacterial viruses. Plenum Press, Cambridge.

Townsend, S.A. 1992. The relationships between salmonellas and faecal indicator concentrations in two pools in the Australian wet/dry tropics. *J. Appl. Microbiol.* 73:182-8.

United Nations/ World Health Organisation. 1996. Assessment of the state of microbiological pollution of the Mediterranean Sea. United Nations Environment Program , Athens.

United States Department of Agriculture, Centers for Epidemiology and Animal Health. 1997. An Update: *Escherichia coli* O157:H7 in Humans and Cattle. USDA, Fort Collins.

United States International Trade Commission. 1993. Live cattle and beef: U.S. and Canadian industry profiles, trade, and factors of competition. USITC, Washington.

Utzet F., R. Lopez, O. Julia, J. Vives-Rego. 1996. Statistical model fitting *Escherichia coli* survival data during starvation in artificial sea water. *Microbios.* 88:45-53

Uyttendaele, M., P. De Troy, J. Debevere. 1999. Incidence of *Salmonella, Campylobacter jejuni, Campylobacter coli,* and *Listeria monocytogenes* in poultry carcasses and different types of poultry products for sale on the Belgian retail market. *J. Food Prot.* 62:735-40.

Vallance, B.A. and B.B. Finlay. 2000. Exploitation of host cells by enteropathogenic *Escherichia coli. Proc. Natl. Acad. Sci.* 97:8799-806.

Van Beneden C.A., W.E. Keene, R.A. Strang, D.H. Werker, A.S. King, B. Mahon, K. Hedberg, A. Bell, M.T. Kelly, V.K. Balan, W.R. Mac Kenzie, D. Fleming. 1999. Multinational outbreak of *Salmonella enterica* serotype Newport infections due to contaminated alfalfa sprouts. *JAMA* 281:158-62

Van Donkersgoed, J., T.Graham, V.Gannon. 1999. The prevalence of verotoxins, *Escherichia coli* O157:H7, and *Salmonella* in the feces and rumen of cattle at processing. *Can. Vet. J.* 40:332-8.

van Gerwen, S.J.C. and M.H. Zwietering. 1998. Growth and inactivation models to be used in quantitative risk assessments. *J. Food Prot.* 61:1541-9.

Van Houten, R., D. Farberman, J. Norton, J. Ellison, J. Kiehlbauch, T. Morris, P. Smith. 1998. *Plesiomonas shigelloides* and *Salmonella* serotype Hartford infections associated with a contaminated water supply-Livingston County, New York, 1996. *MMWR*. 47:394-6.

Wang, G., M.P. Doyle. 1998. Survival of enterohemorrhagic *Escherichia coli* O157:H7 in water. *J. Food Prot.* 61:662-7.

Warburton, D.W., B.Bowen, A. Konkle. 1994. The survival and recovery of *Pseudomonas aeruginosa* and its effect upon salmonellae in water: methodology to test bottled water in Canada. *Can. J Microbiol.* 40:987-92.

Warburton, D.W., J.W. Austin, B.H. Harrison, G. Saunders. 1998. Survival and recovery of *Escherichia coli* O157:H7 in inoculated bottled water. *J. Food Prot.* 61:948-52.

Warmer, M., K. Kuo, L. Williams, B.Adam, C. Langkop, R. Ruden, B. Francis, T. Haupt. 1996. Lake-associated outbreak of *Escherichia coli* O157:H7-Illinois, 1995. *MMWR* 45:5-7.

Waters, J.R., J.C. Sharp, V.J. Dev. 1994. Infection caused by *Escherichia coli* O157:H7 in Alberta, Canada, and in Scotland: A five-year review, 1987-1991. *Clin. Infect. Dis.* 19:834-43.

Whiting, R.C. 1993. Modeling bacterial survival in unfavourable environments. *J. Ind. Microbiol.* 12:240-6.

Whiting, R.C., R.L. Buchanan. 1997. Predictive modeling p. 728-39. *In*: Doyle, M.P., L.R. Beuchat, T.J. Montville (eds) Food microbiology: fundamentals and frontiers. ASM Press, Washington, D.C.

Whiting, R.C., S. Sackitey, S. Calderone, K. Morely, J.G. Phillips. 1996. Model for the survival of *Staphylococcus aureus* in nongrowth environments. *Int. J. Food Microbiol.* 31:231-43.

Willshaw, G.A., H.R. Smith, S.M. Scotland, A.M. Field, B. Rowe. 1987. Heterogeneity of *Escherichia coli* phages encoding Vero cytotoxins: comparison of cloned sequences determining VT1 and VT2 and development of specific gene probes. *J. Gen. Microbiol.* 133:1309-17.

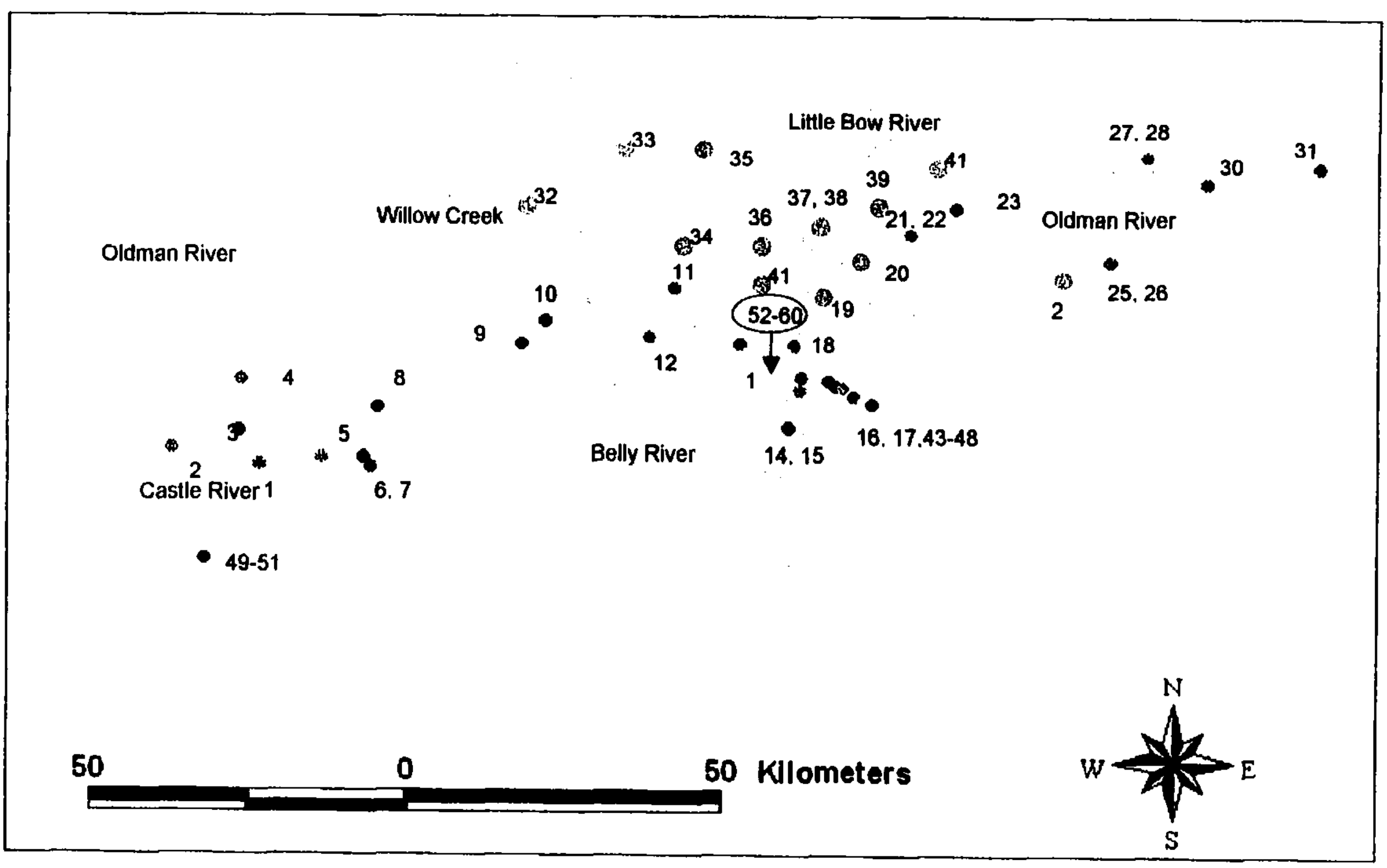
Willshaw, G.A., S.M. Scotland, and B. Rowe. 1997. Vero-cytotoxin-producing *Escherichia coli*. p.421-48. *In* Sussman, M.(ed) *Escherichia coli*: Mechanisms of virulence. Cambridge, Cambridge University Press.

Xiong, R., G.Xie, A.E. Edmondson, M.A. Sherard. 1999. A mathematical model for bacterial survival. *Int. J. Food. Microbiol.* 46:45-55.

Zhao, T., M.P. Doyle, J.Shere, L Garber. 1995. Prevalence of enterohemorrhagic *Escherichia coli* O157:H7 in a survey of dairy herds. *Appl. Environ. Microbiol.* 61:1290-3.

Appendices

Appendix 1 Prevalence Study regularly sampled Sites 1-60, 1999 and 2000, site names on following pages



1 Castle River

2 Crowsnest River, Coleman

3 Crowsnest River, Connelly Creek

4 Oldman River , Olin Creek

5 Oldman River , bottom release

6 Oldman River , Brocket

7 Pincher Creek

8 Beaver Creek

9 Oldman River, Fort MacLeod

10 Willow Creek

11 Oldman River, Monarch

12 Belly River

13 Oldman River, Belly River

14 Haney drain

15 St Mary River

16 Oldman River, Popson Park

17 Six Mile Coulee spillway

18 Oldman River, Hwy 3 Bridge

19 Oldman River, Diamond City

20 Piyami Drain

21 Oldman River , Picture Butte

22 Battersea Drain

23 Little Bow River

24 Bountiful Coulee

25 Drain T-2

26 Oldman River, Taber

27 Oldman River, Hwy 36 Bridge

28 Expanse Coulee

29 Drain T 11

30 Oldman River , Purple Springs

31 Oldman River, mouth

32 LNID main Canal

33 LNID Barons intake

34 LNID, Nobleford intake

35 LNID, Keho Lake outlet

36 LNID, Shaughnessy intake

37 Inlet to Picture Butte Reservoir

38 Picture Butte Reservoir Outlet

39 LNID Iron Springs intake

40 LNID, Turin intake

41 LNID, Park Lake

42 6-Mile Coulee, storm drain

43 6-Mile Coulee, natural drain

44 6-Mile Coulee, Whoop-up Spillway

45 6-Mile Coulee, Tiffin drain

46 6-Mile Coulee, Carlson Livestock

47 6-Mile Coulee, Santangelo Farm

48 6-Mile Coulee, Main Branch

49 West Castle River above ski hill

50 West Castle River below ski hill

51 West Castle River, Castle River

52 Six Mile Coulee, Storm Drain 94-
S8

53 Storm Drain S-7

54 Storm Drain 94-S8

55 Storm Drain S-5/6

56 Storm Drain S-3

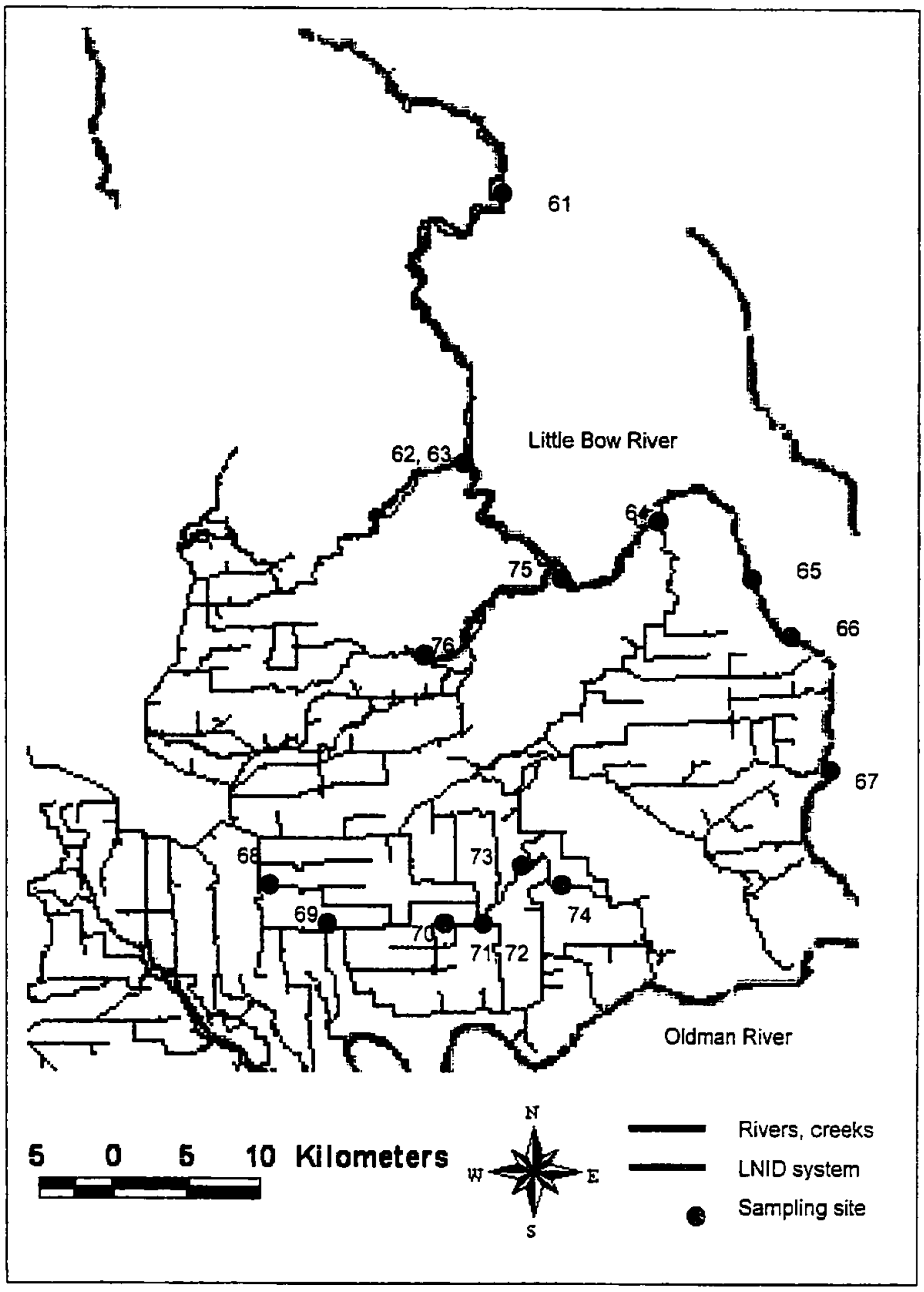
57 Storm Drain N-2

58 Storm Drain W-3

59 Storm Drain, University pond

60 Storm Drain, Churchill Storm Pond

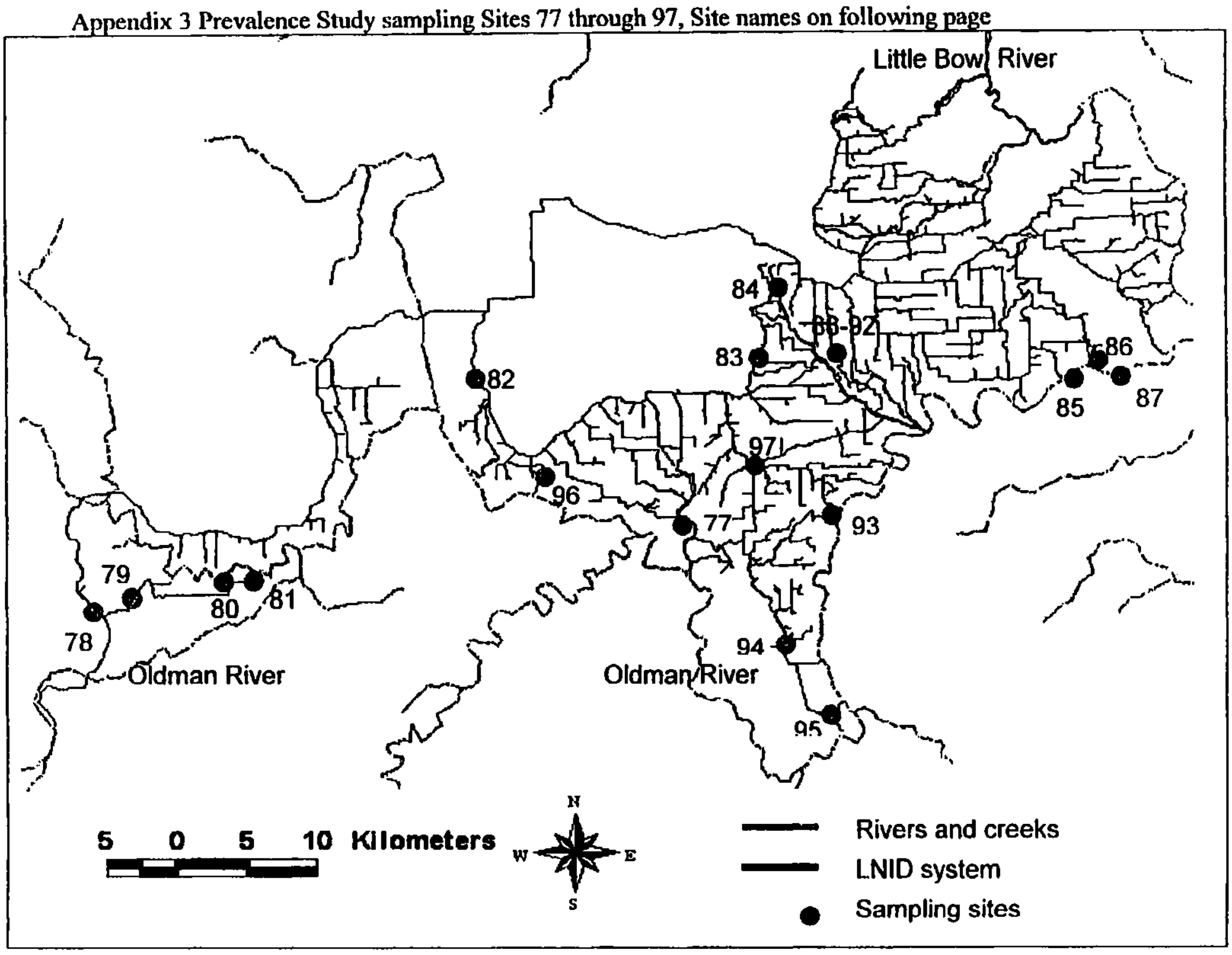
Appendix 2 Prevalence Study Sites 61 through 76, Site names on following page



61	LB2M
62	LB3M
63	LB4-2P
64	LB4M
65	LB5-10
66	LB5M
67	LB6-9A
68	B1M
69	B2M
70	B4-4L
71	B4-5M
72	B4M
73	B5M
74	B6M
75	S1DC
76	S2DC

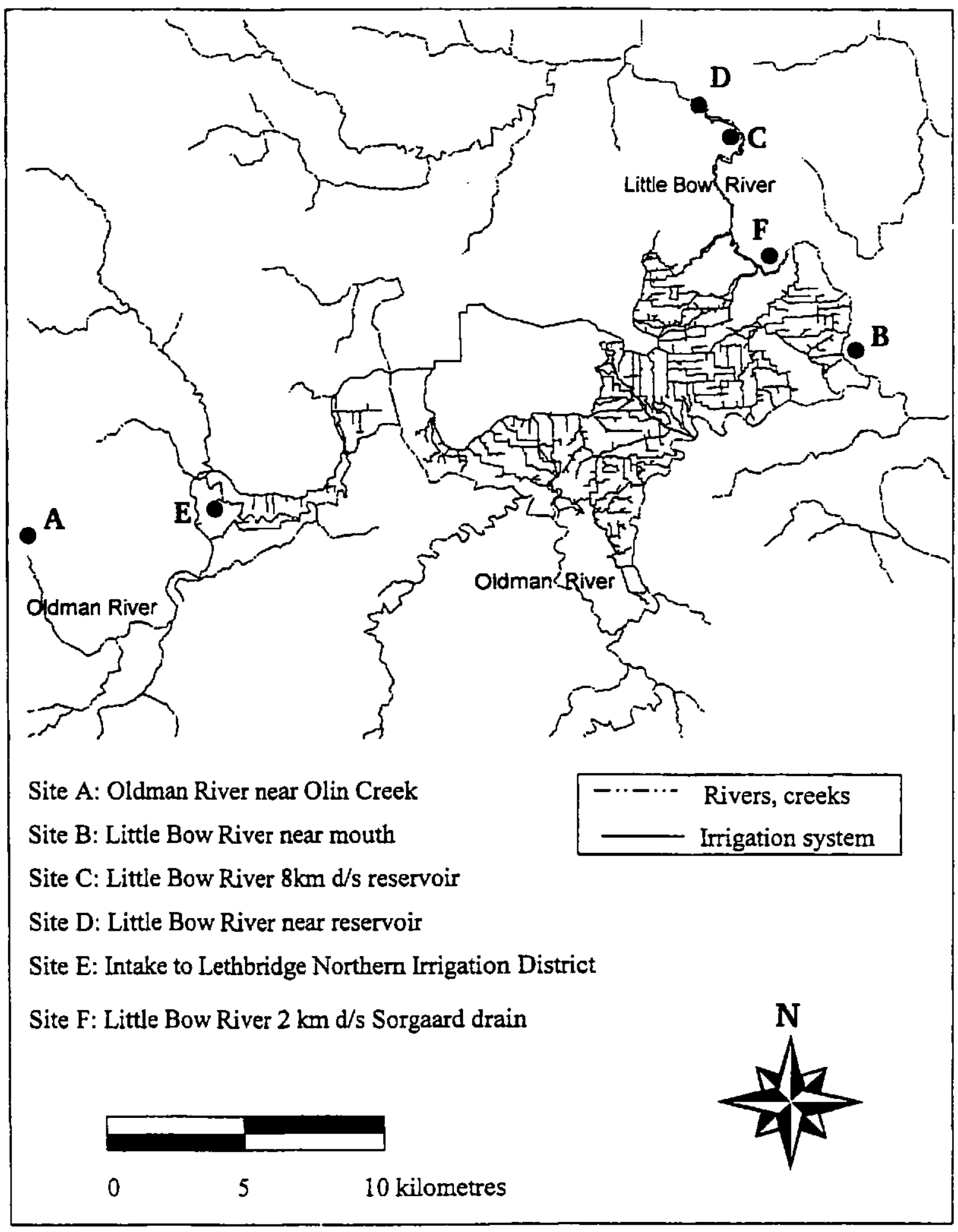
LB= Little Bow River; B = Battersea drain; M = main; P = Pitchfork drain; A= Arrowhead coulee; SDC = Sorgaard drain; L = lateral

Appendix 3 Prevalence Study sampling Sites 77 through 97, Site names on following page

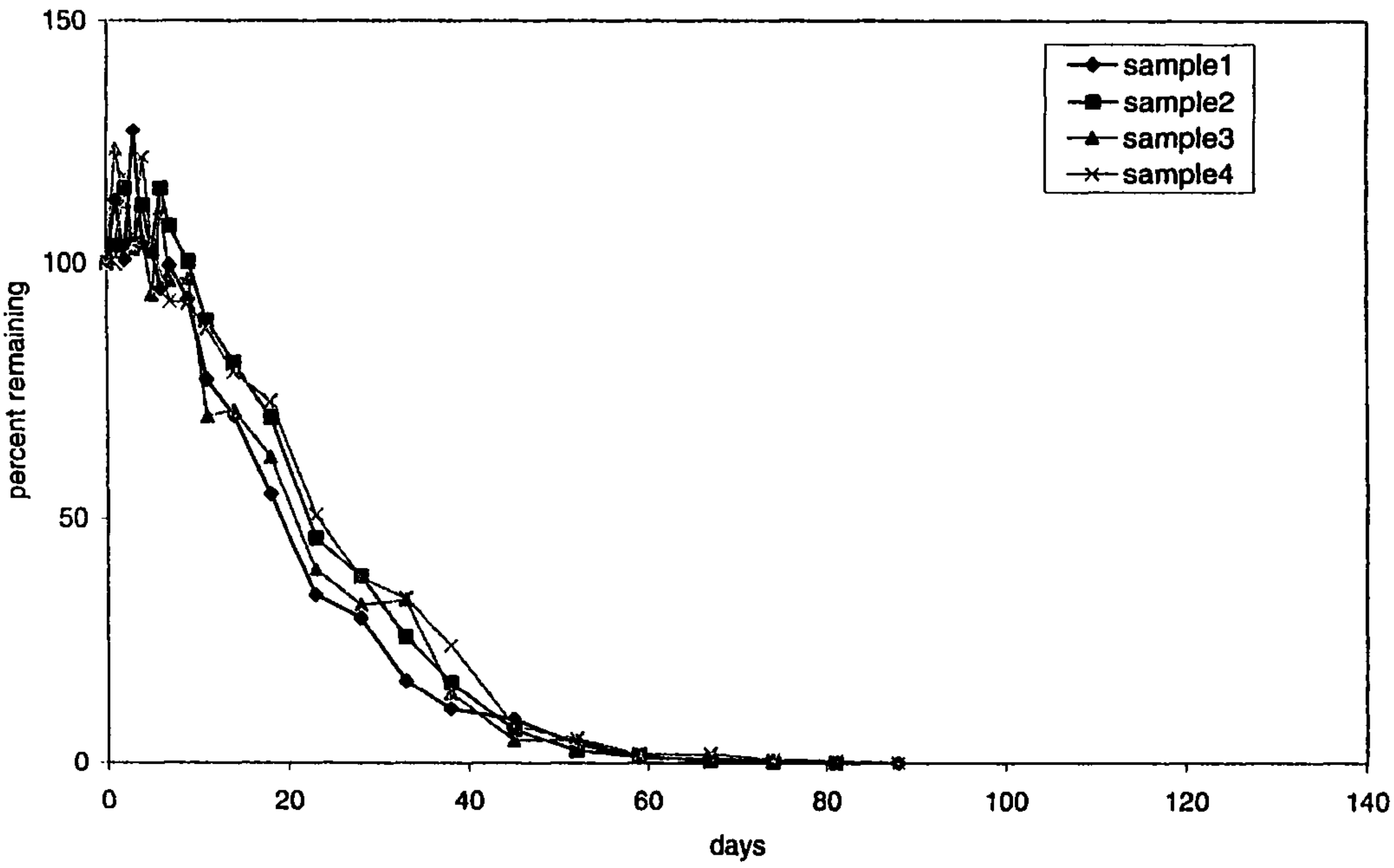


77	Monarch drain
78	A-1 inflow
79	A-1 at Highway 2
80	A-1 at McIntosh section
81	A-1 outflow
82	Monarch main
83	Piyami at Highway 519
84	E-6 outflow
85	Oldman River upstream of Battersea drain
86	Battersea drain
87	Oldman River downstream of Battersea drain
88	Park Lake inflow
89	Park Lake north
90	D1 inflow
91	Park Lake south
92	61C inlet (Park Lake south outlet)
93	Szteina drain
94	West side inlet
95	West side outlet
96	West Monarch drain
97	F-6 structure

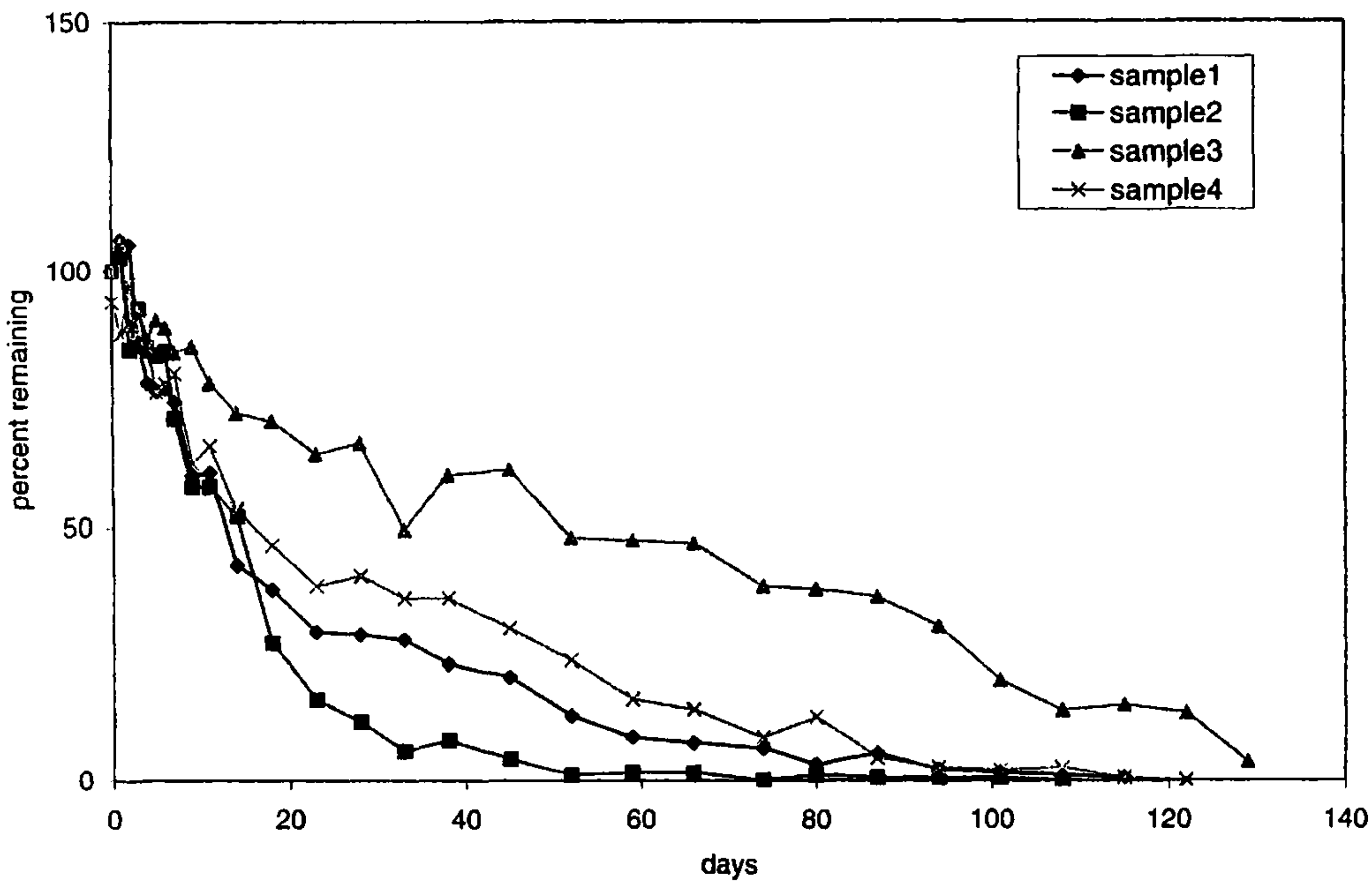
Appendix 4. Sites of water collected for survival study.



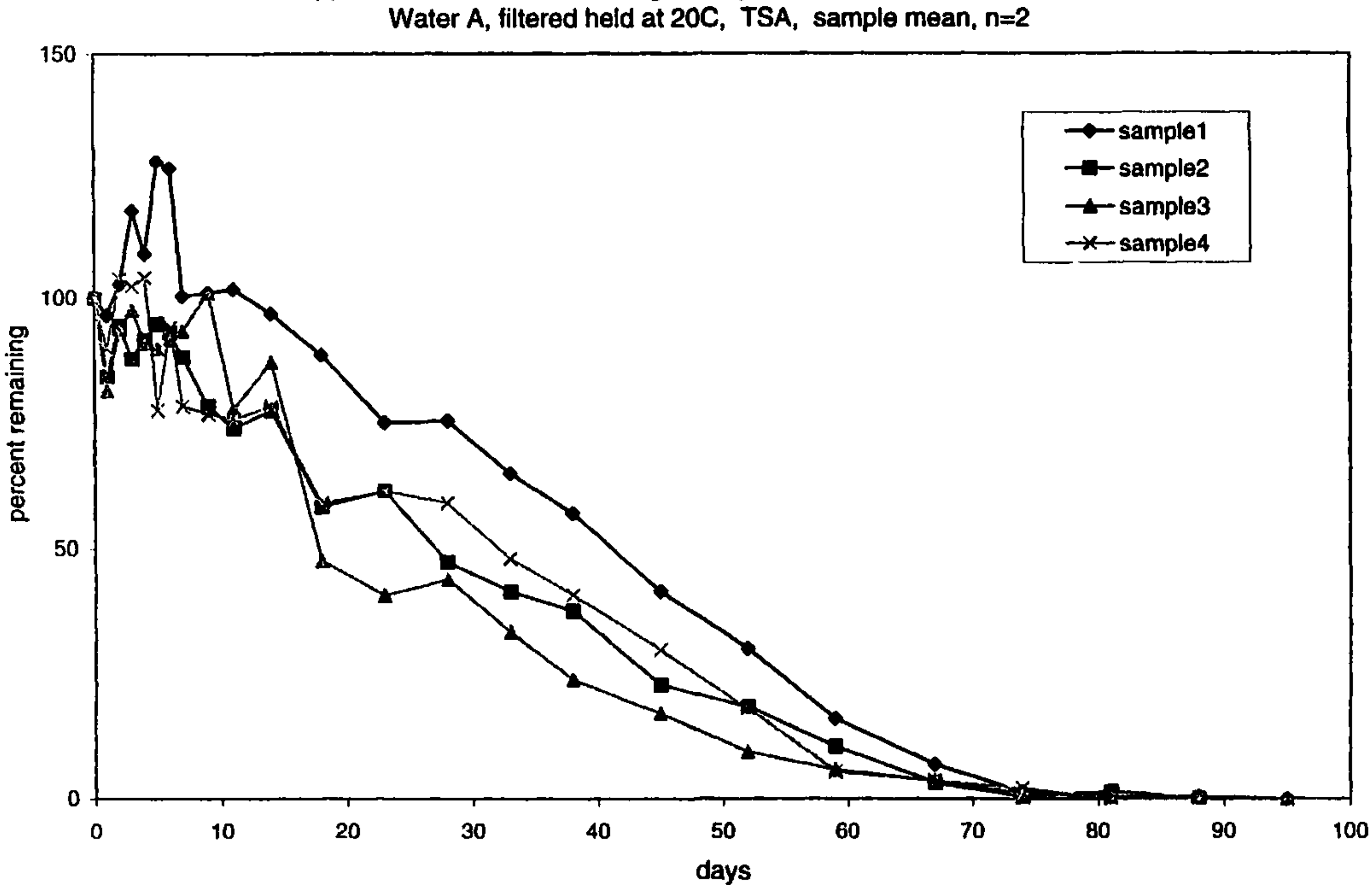
Appendix 5. Percent remaining of S. typhimurium waterborne isolate in Water A , filtered held at 10C, TSA, sample mean, n=2



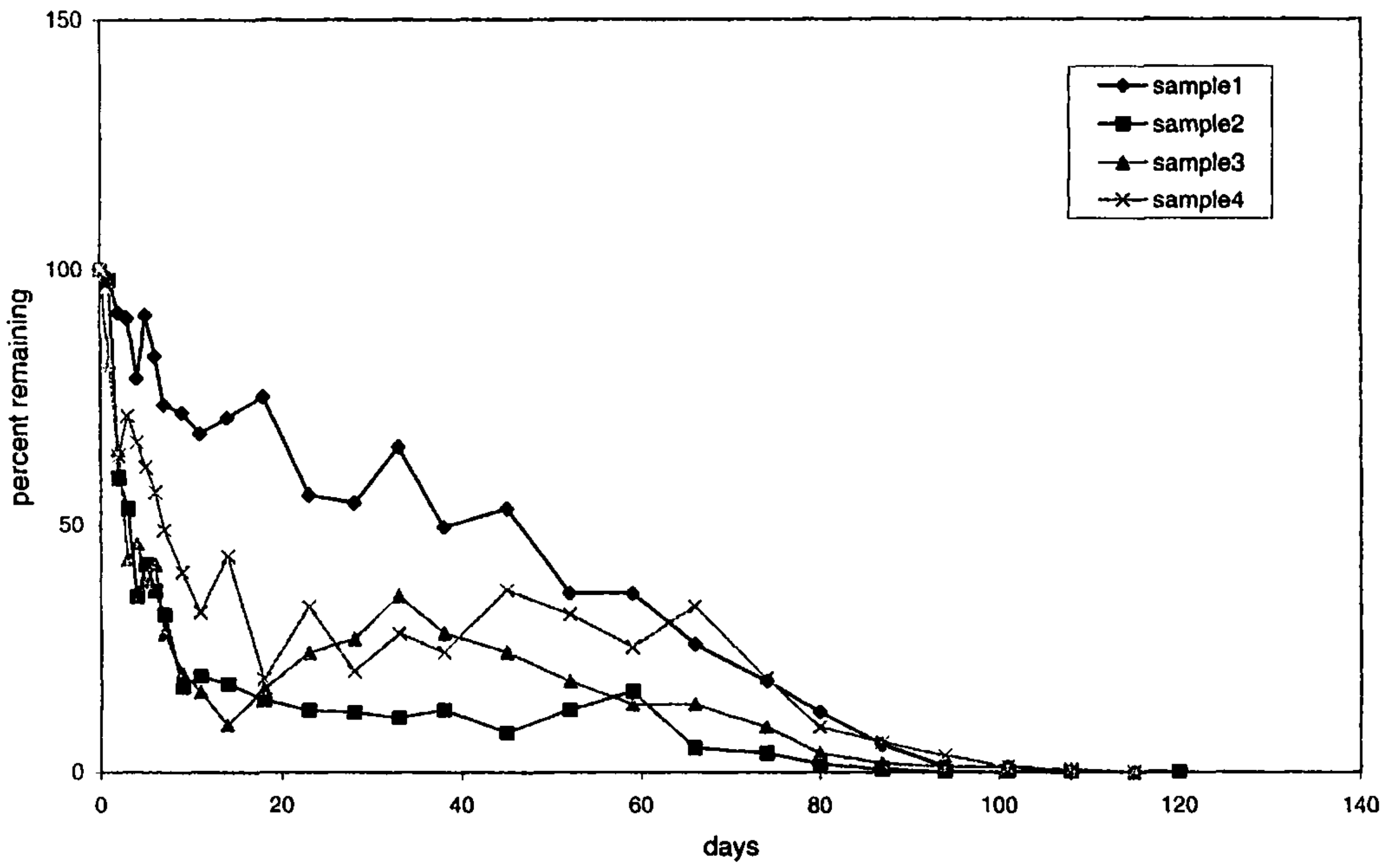
Appendix 6. Percent remaining of E. coliO157:H7 waterborne isolate in
Water A, filtered held at 10C, TSA, sample mean, n=2



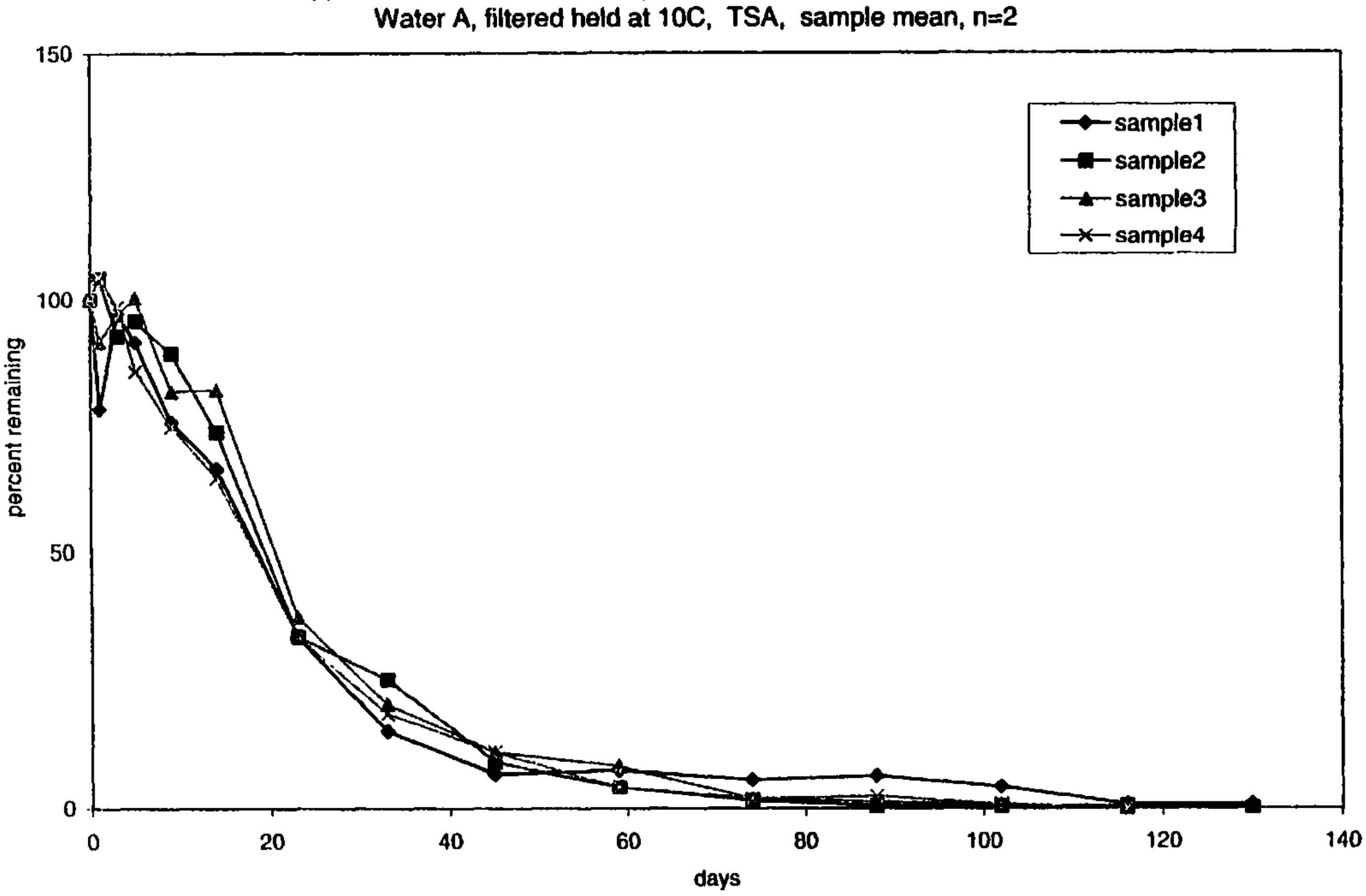
Appendix 7. Percent remaining of S. typhimurium waterborne isolate in Water A, filtered held at 20C, TSA, sample mean, n=2



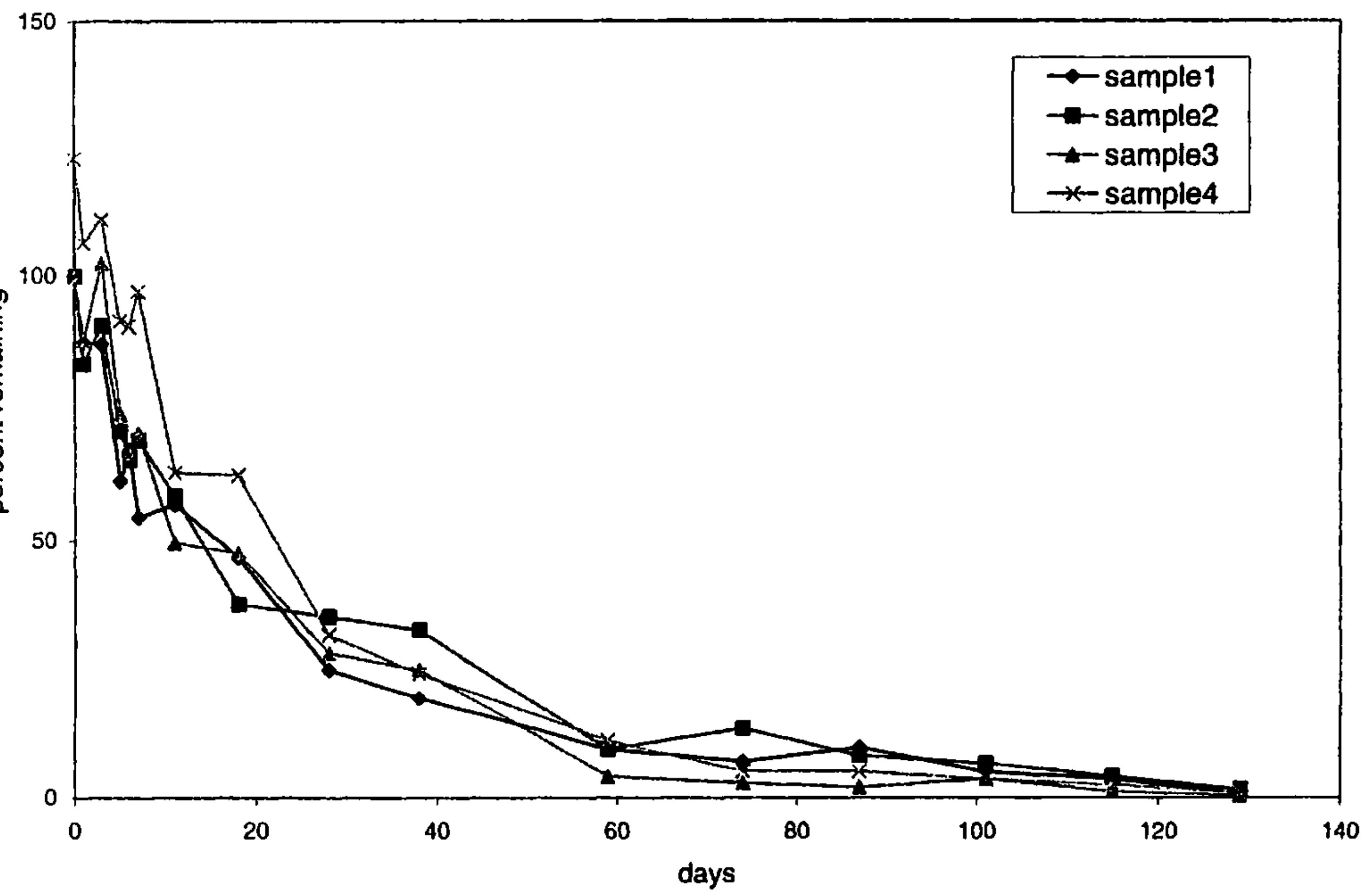
Appendix 8. Percent remaining of E. coliO157:H7 waterborne isolate in Water A, filtered held at 20C, TSA, sample mean, n=2



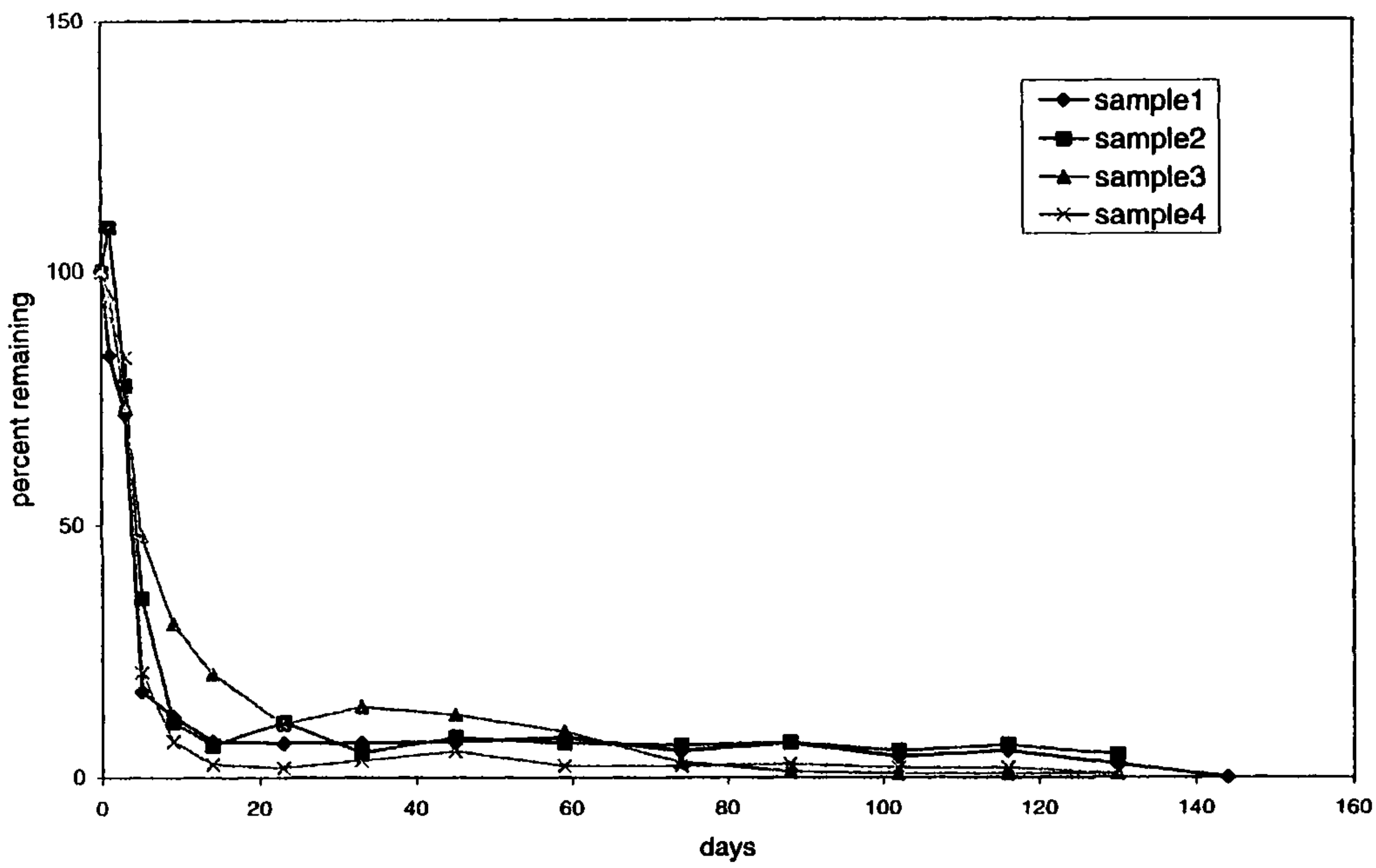
Appendix 9. Percent remaining of S. typhimurium laboratory isolate in Water A, filtered held at 10C, TSA, sample mean, n=2



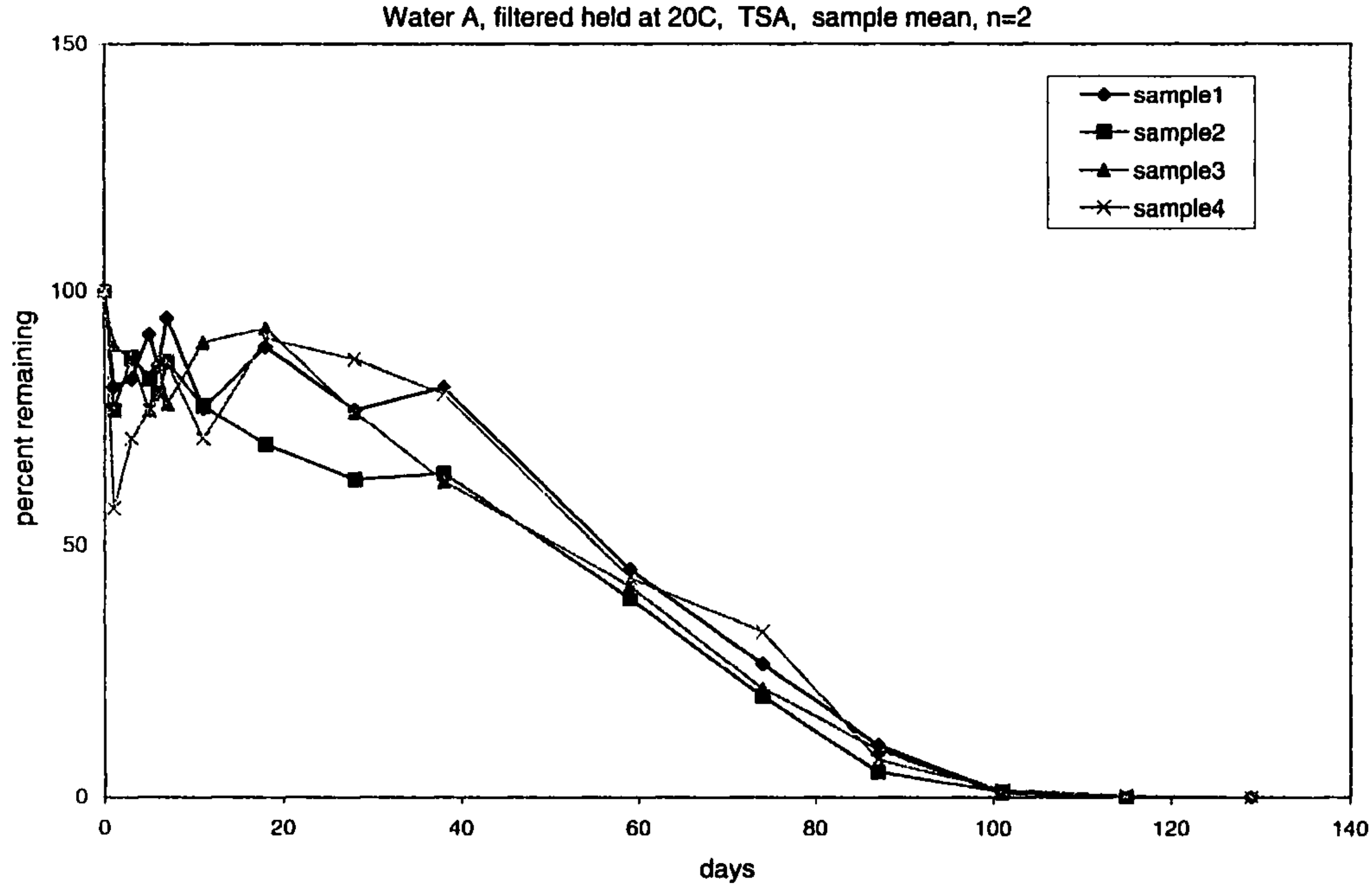
Appendix 10. Percent remaining of E. coliO157:H7 bovine isolate in Water A, filtered held at 10C, TSA, sample mean, n=2



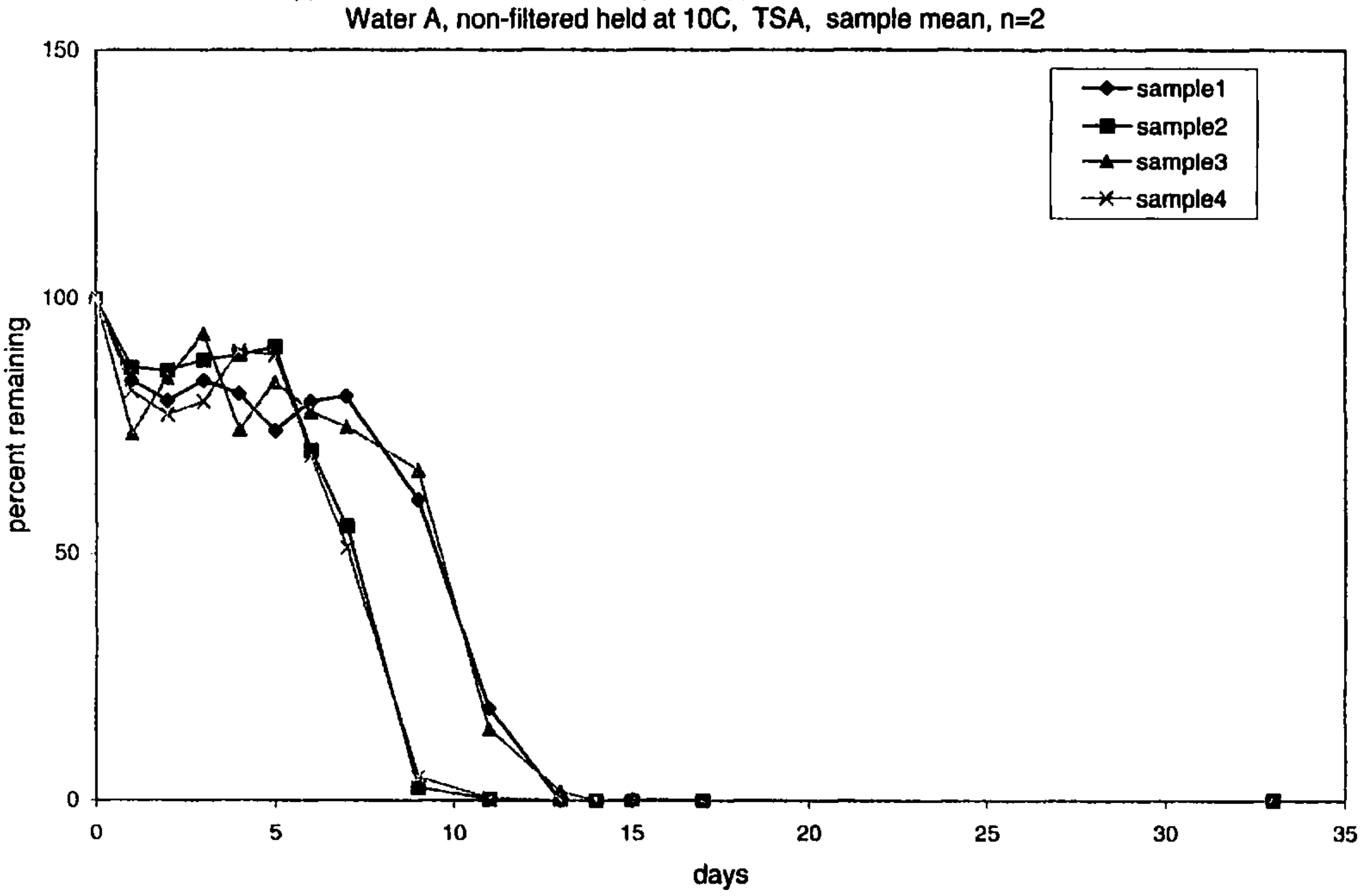
Appendix 11. Percent remaining of S. typhimurium laboratory isolate in Water A, filtered held at 20C, TSA, sample mean, n=2



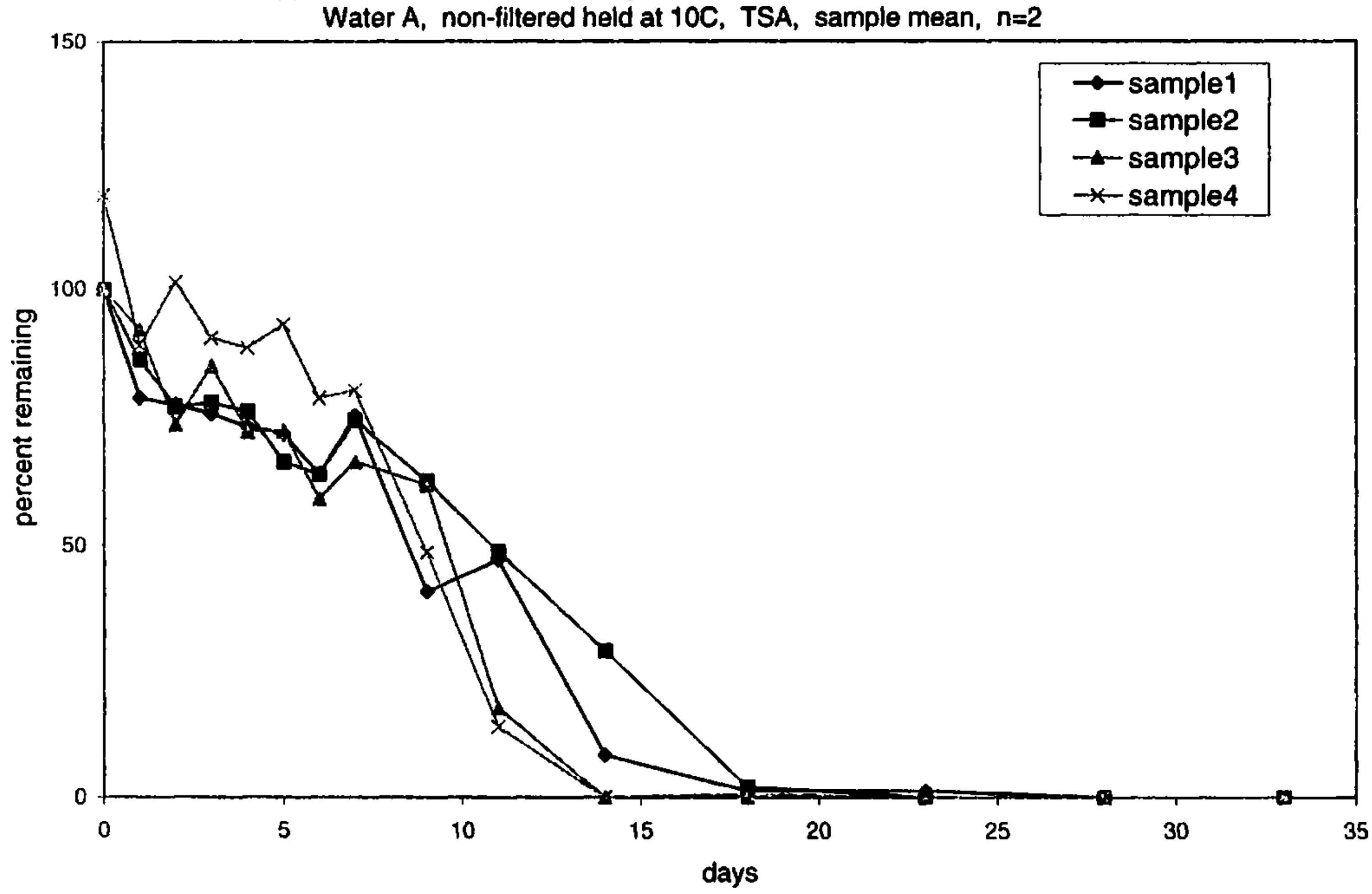
Appendix 12. Percent remaining of E. coliO157:H7 bovine isolate in Water A, filtered held at 20C, TSA, sample mean, n=2



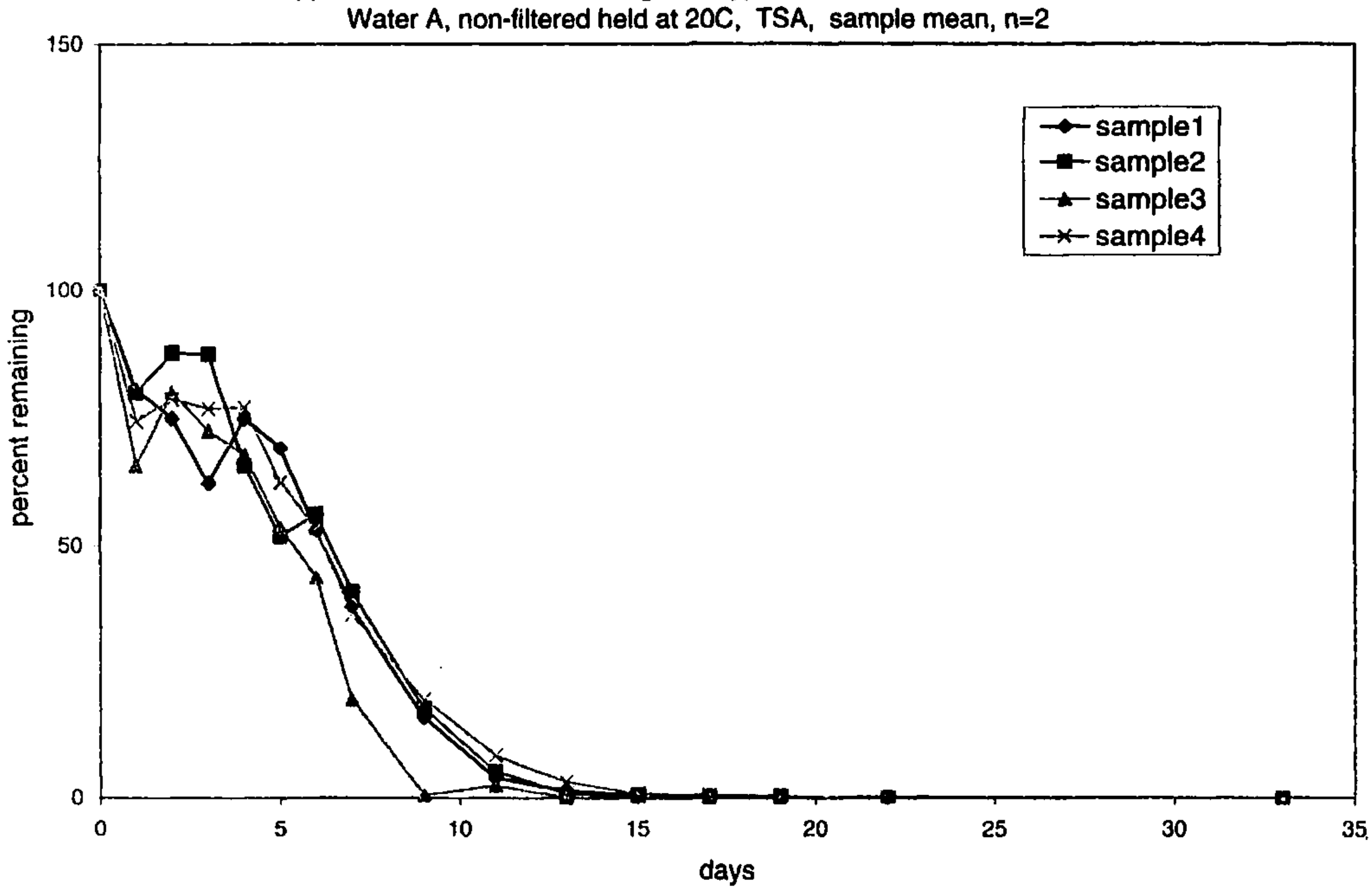
Appendix 13. Percent remaining of S. typhimurium waterborne isolate in Water A, non-filtered held at 10C, TSA, sample mean, n=2



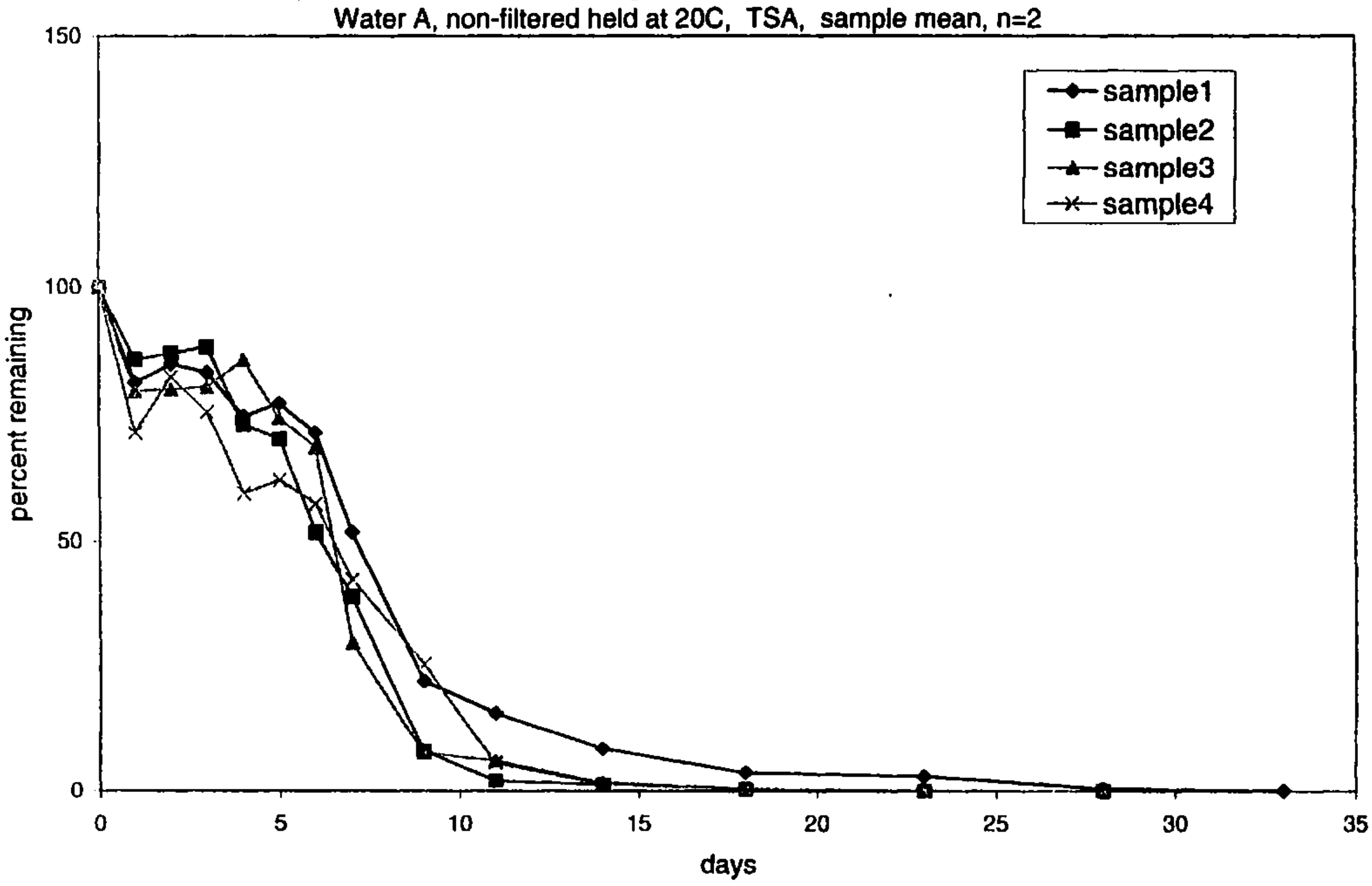
Appendix 14. Percent remaining of E. coliO157:H7 waterborne isolate in Water A, non-filtered held at 10C, TSA, sample mean, n=2



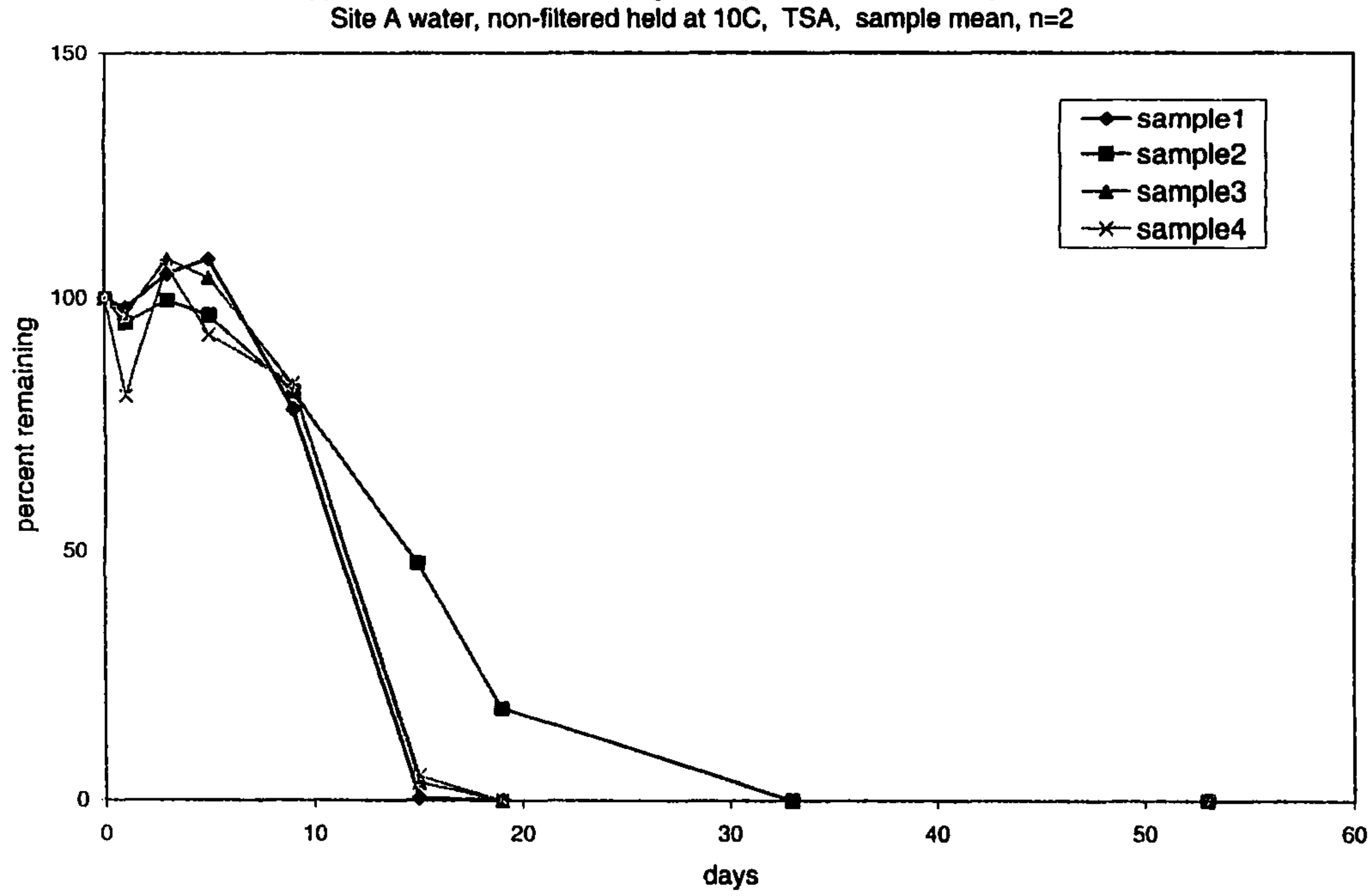
Appendix 15. Percent remaining of S. typhimurium waterborne isolate in Water A, non-filtered held at 20C, TSA, sample mean, n=2



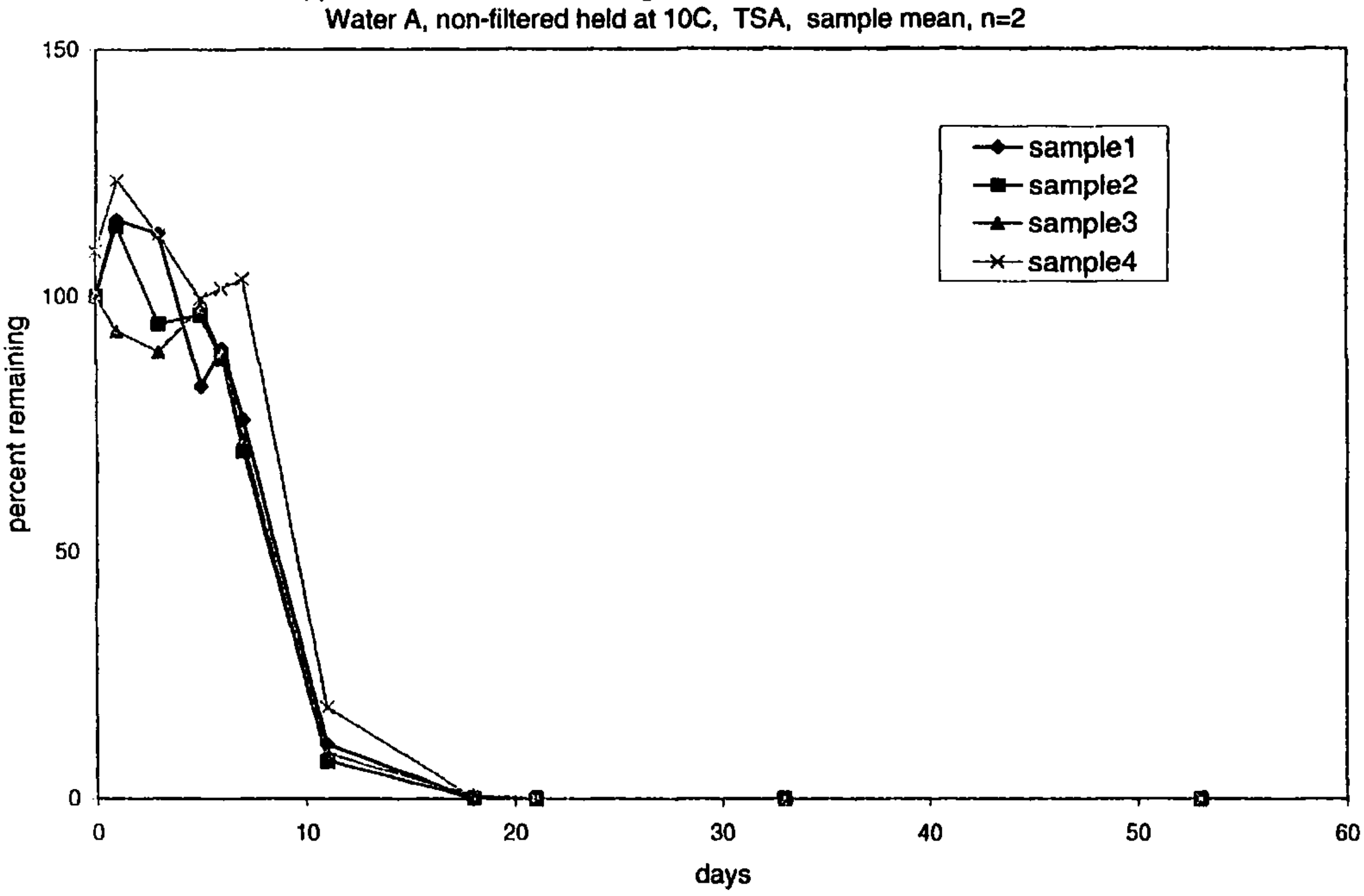
Appendix 16. Percent remaining of E. coliO157:H7 waterborne isolate in Water A, non-filtered held at 20C, TSA, sample mean, n=2



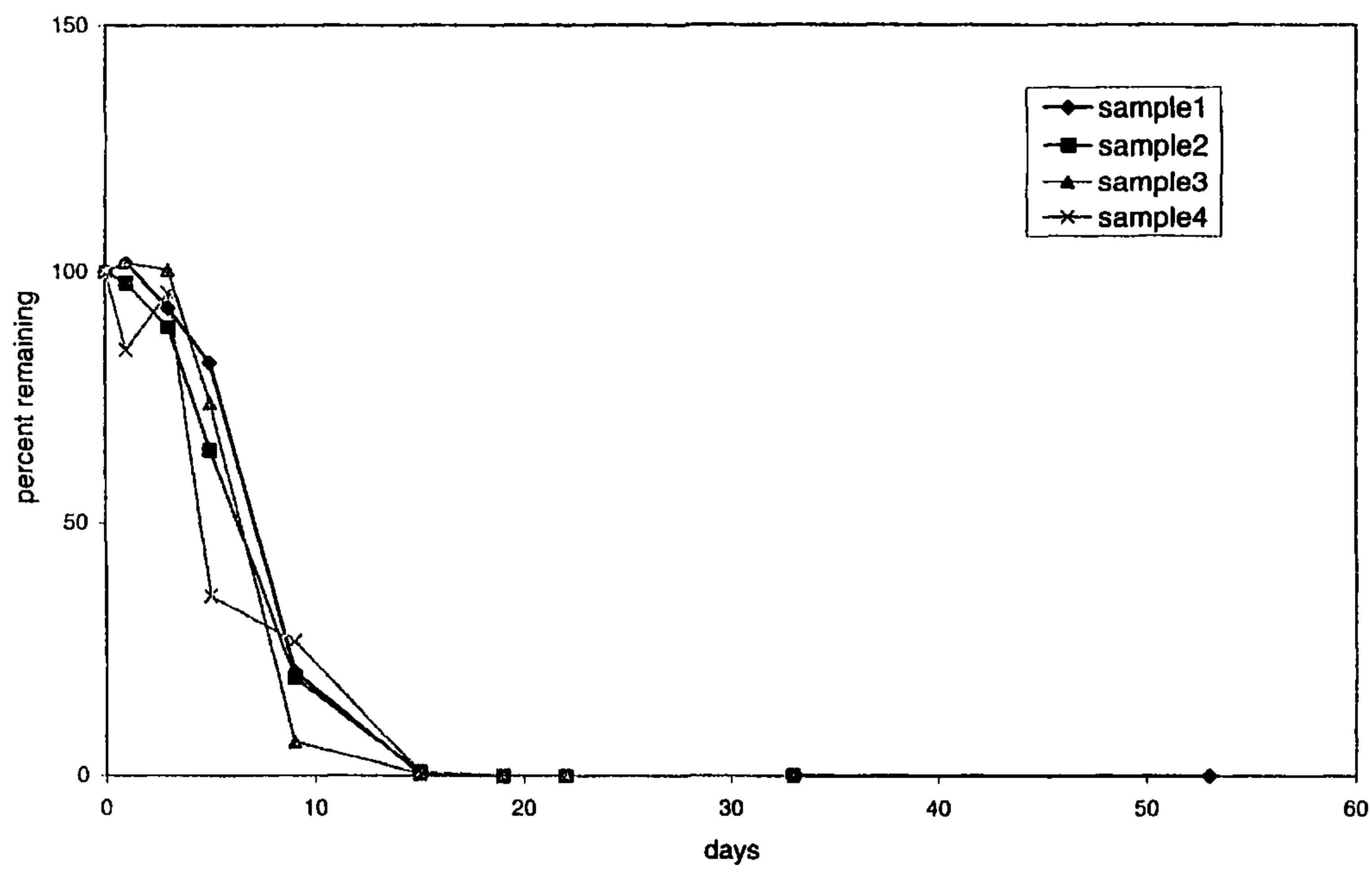
Appendix 17. Percent remaining of S. typhimurium laboratory isolate in Site A water, non-filtered held at 10C, TSA, sample mean, n=2



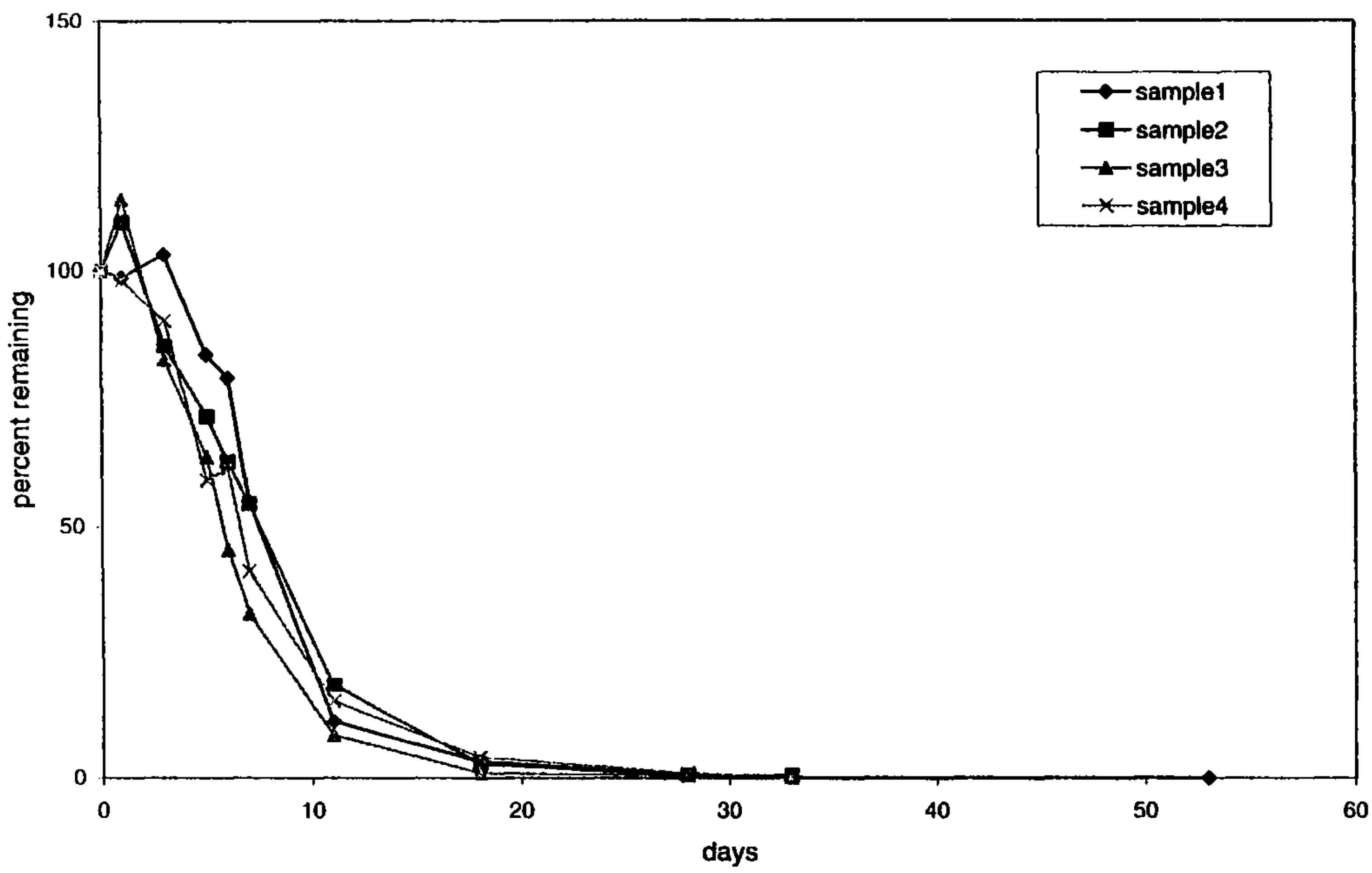
Appendix 18.Percent remaining of E. coliO157:H7 bovine isolate in
Water A, non-filtered held at 10C, TSA, sample mean, n=2



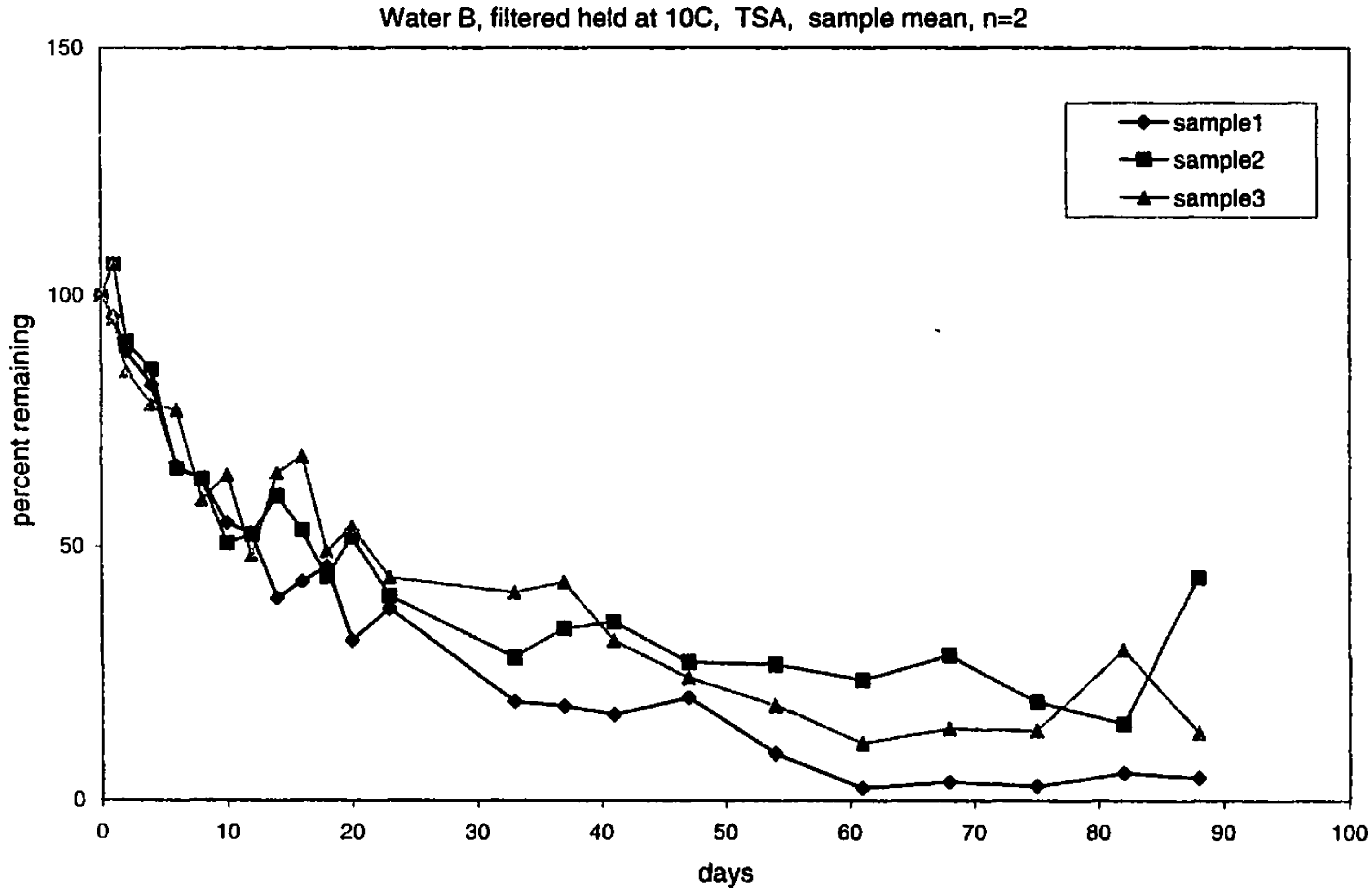
Appendix 19.Percent remaining of S. typhimurium laboratory isolate in Water A, non-filtered held at 20C, TSA, sample mean, n=2



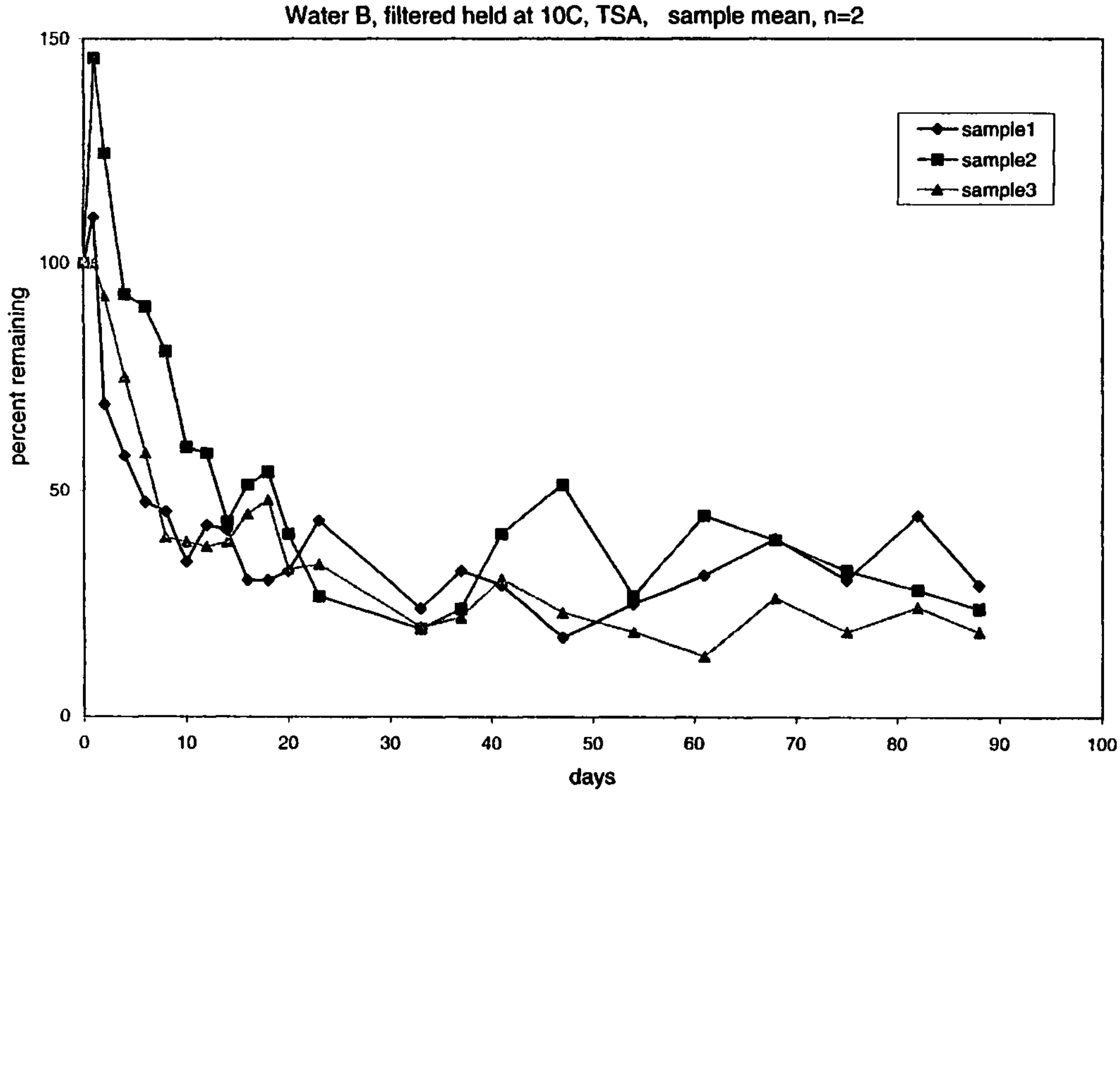
Appendix 20.Percent remaining of E. coliO157:H7 bovine isolate in Water A, non-filtered held at 20C, TSA, sample mean, n=2



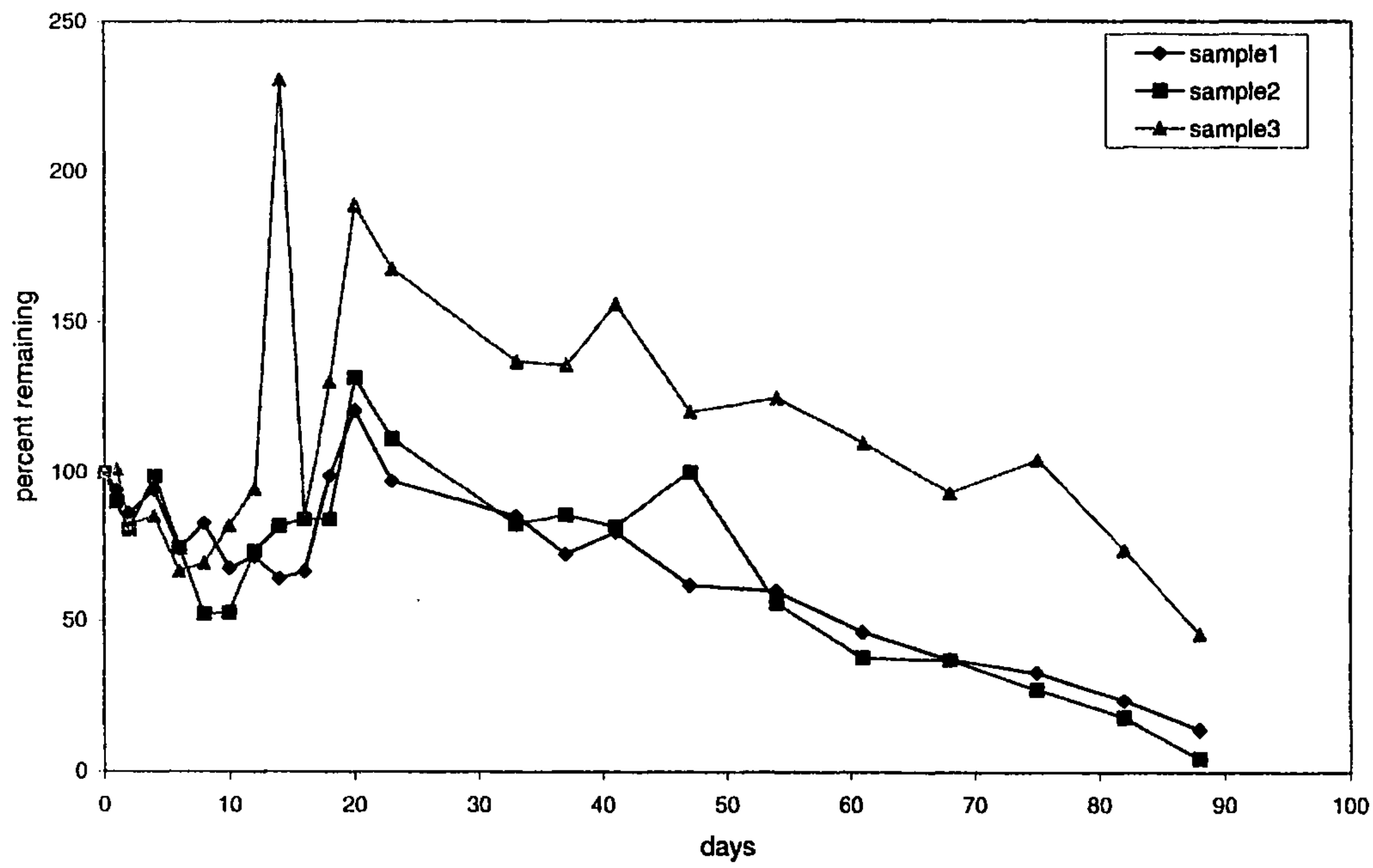
Appendix 21.Percent remaining of S. typhimurium waterborne isolate in Water B, filtered held at 10C, TSA, sample mean, n=2



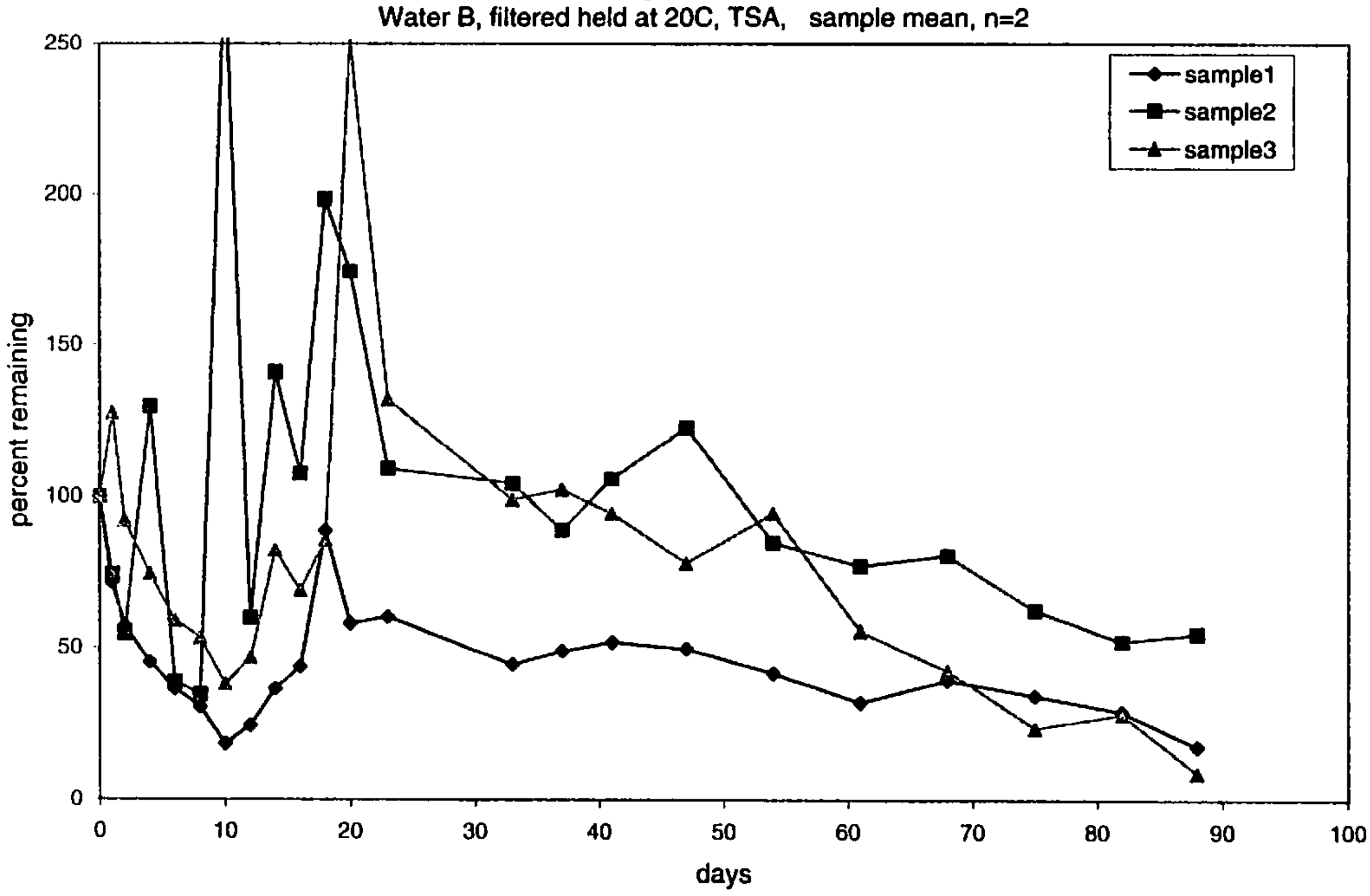
Appendix 22.Percent remaining of E. coliO157:H7 waterborne isolate in Water B, filtered held at 10C, TSA, sample mean, n=2



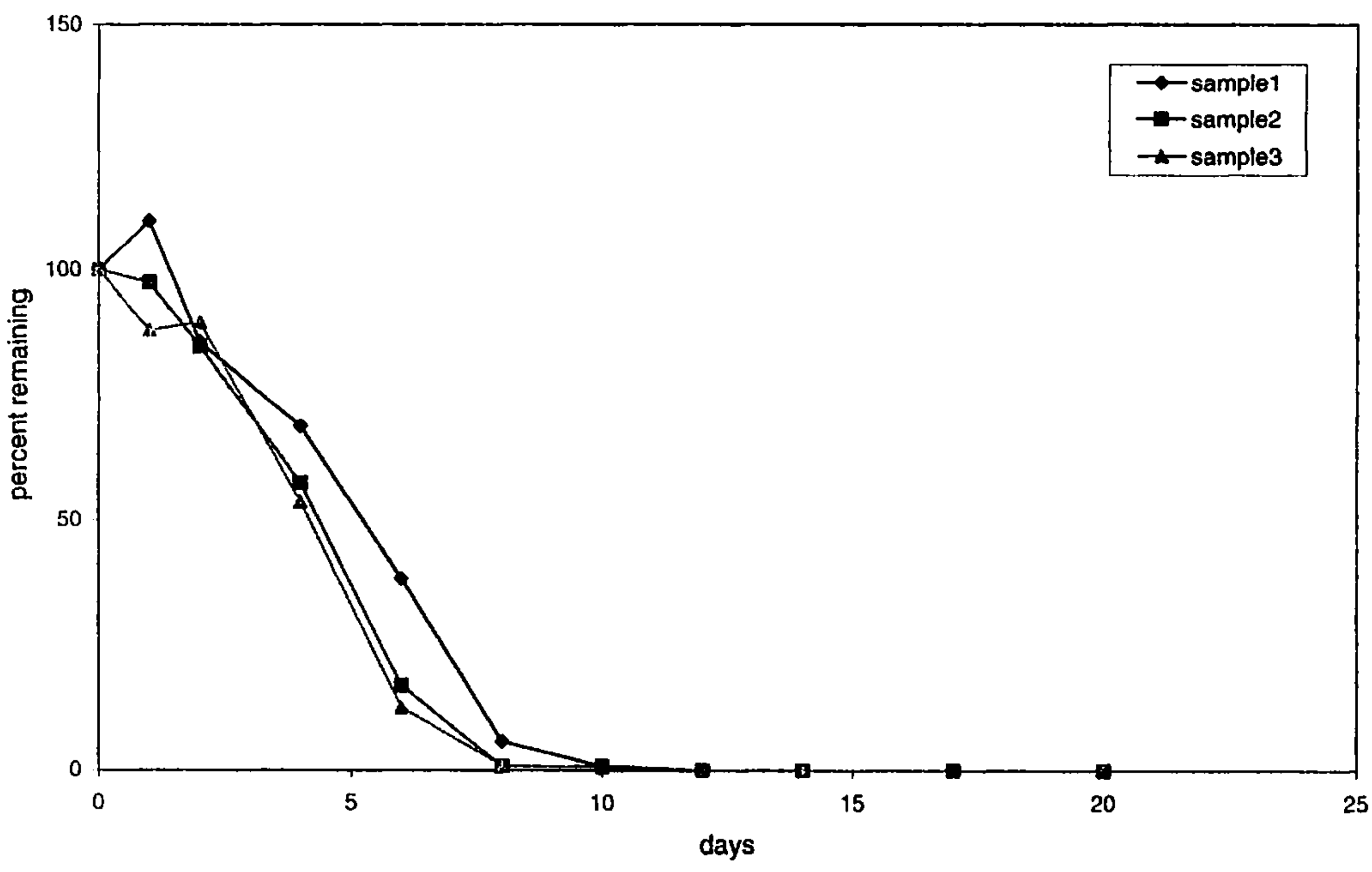
Appendix 23.Percent remaining of S. typhimurium waterborne isolate in Water B, filtered held at 20C, TSA, sample mean, n=2



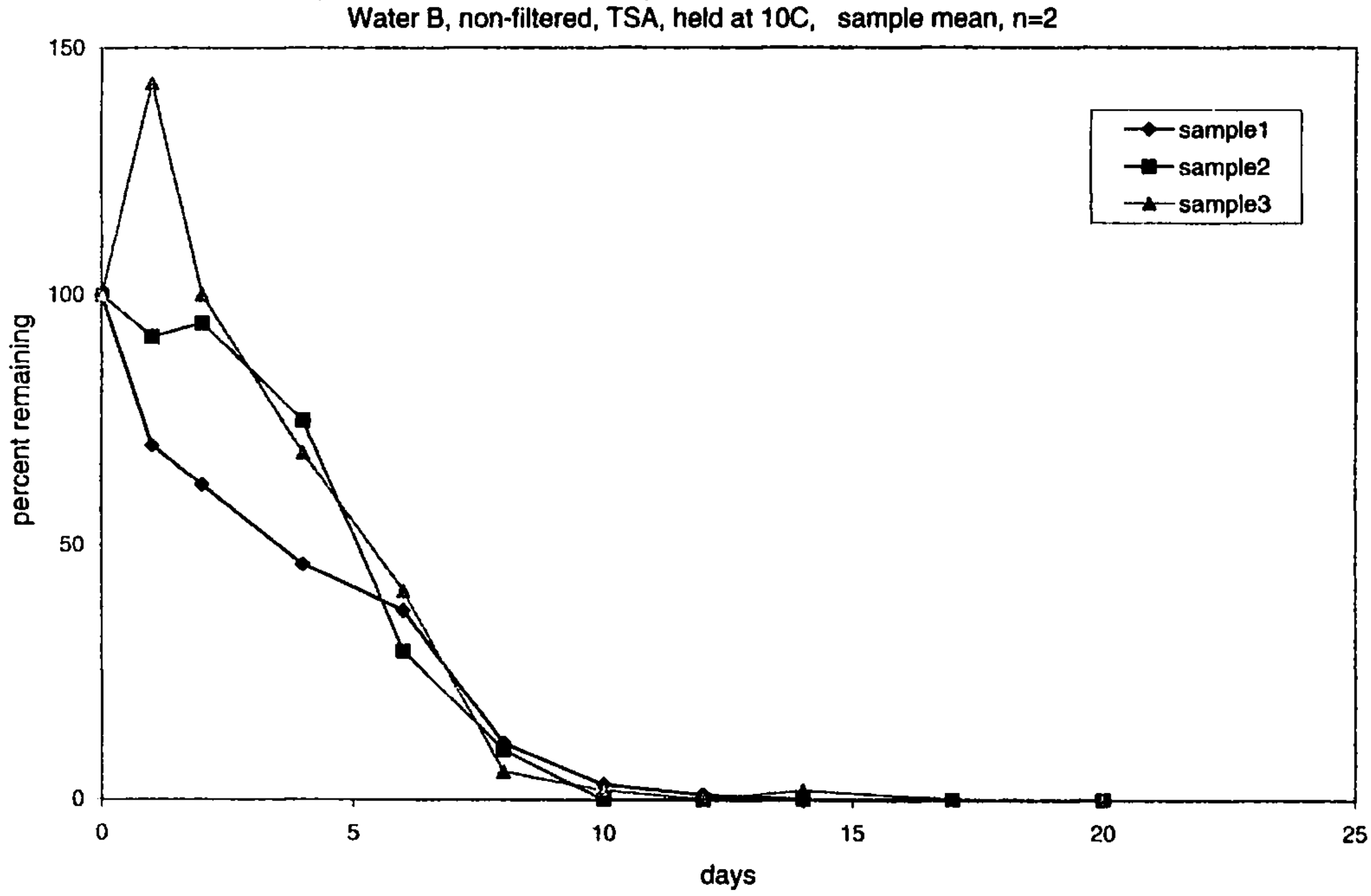
Appendix 24.Percent remaining of E. coliO157:H7 waterborne isolate in Water B, filtered held at 20C, TSA, sample mean, n=2



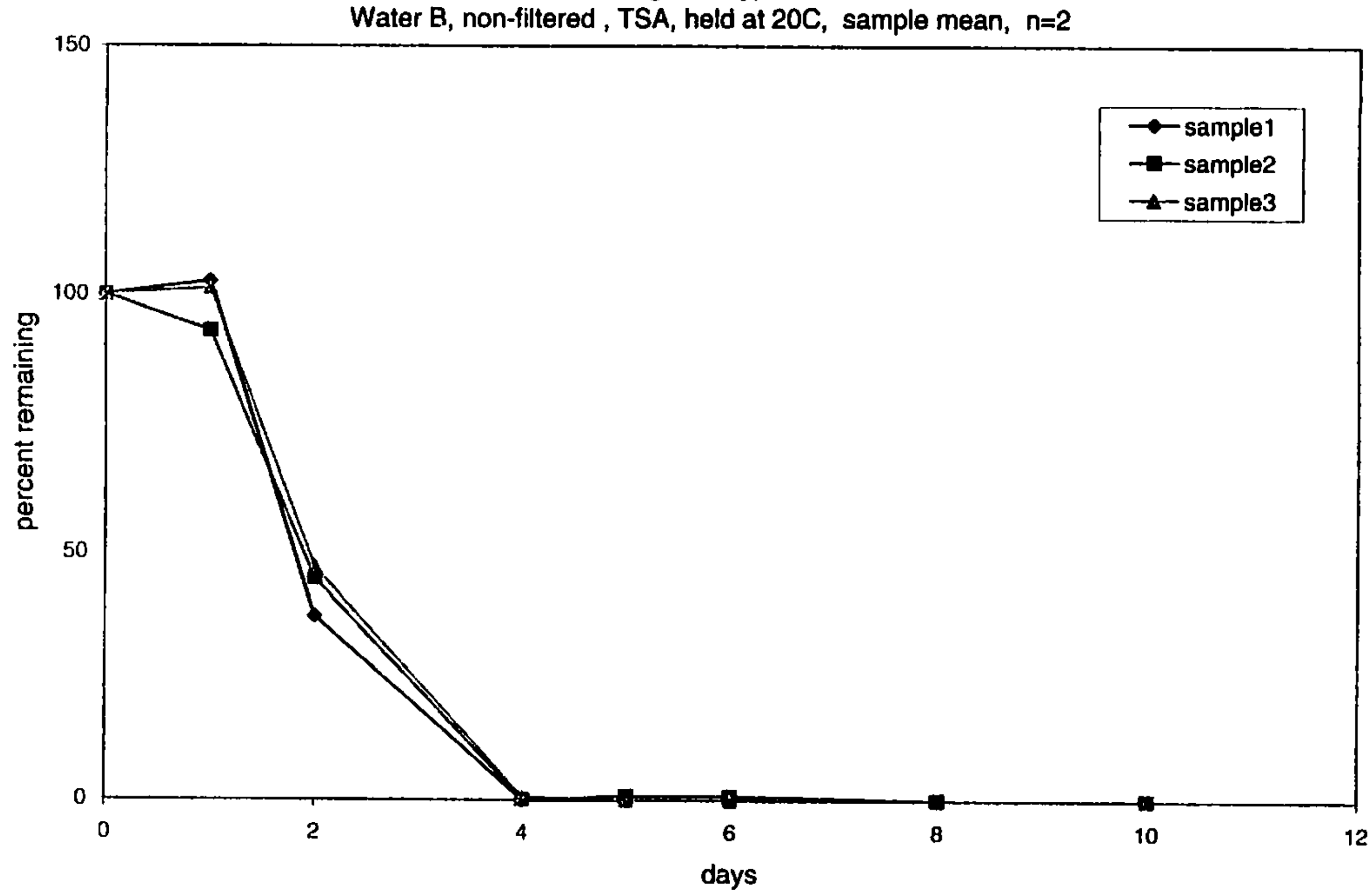
Appendix 25.Percent remaining of S. typhimurium waterborne isolate in Water B, non-filtered, TSA, held at 10C, sample mean, n=2



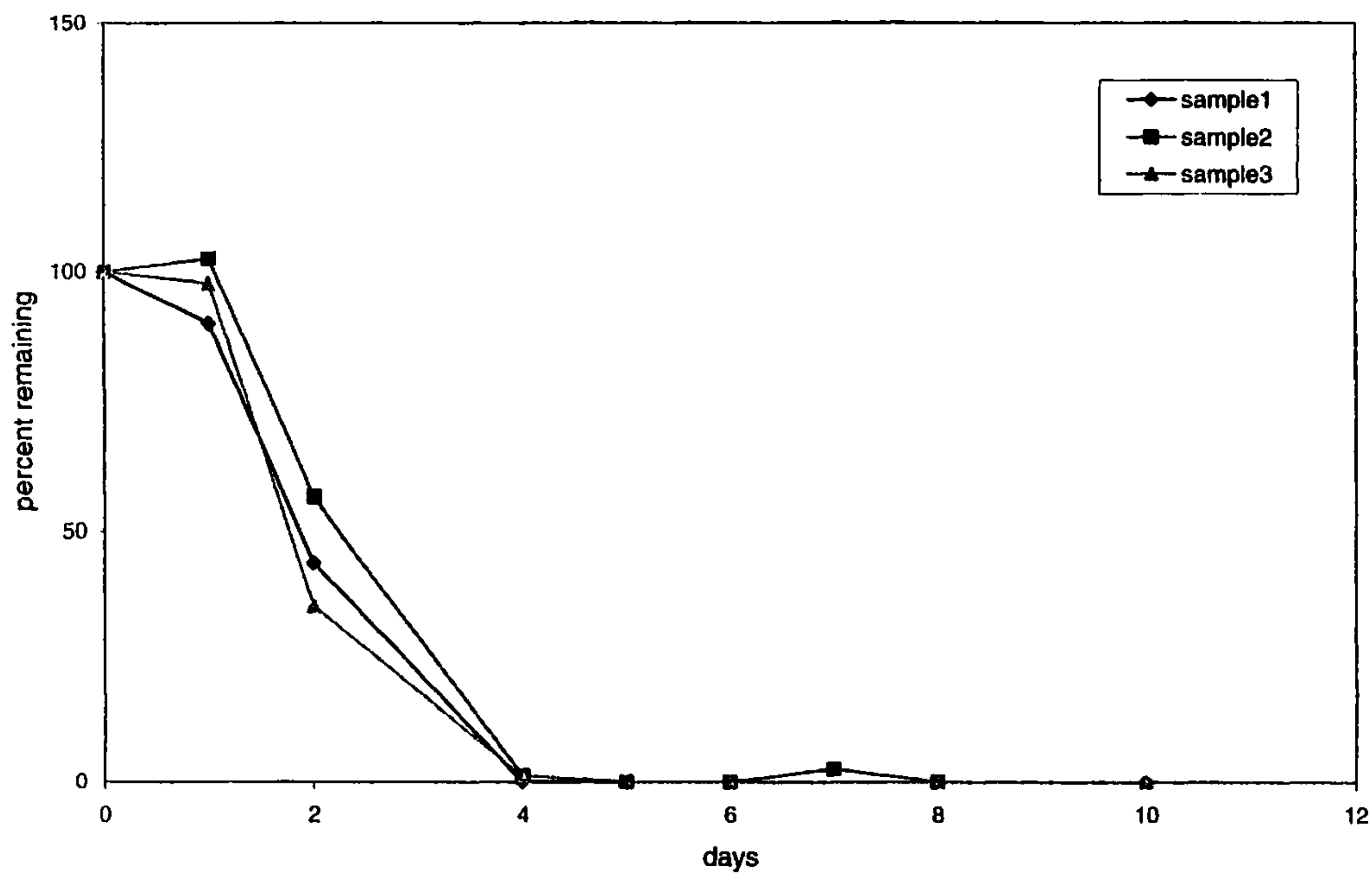
Appendix 26.Percent remaining of E. coliO157:H7 waterborne isolate in Water B, non-filtered, TSA, held at 10C, sample mean, n=2



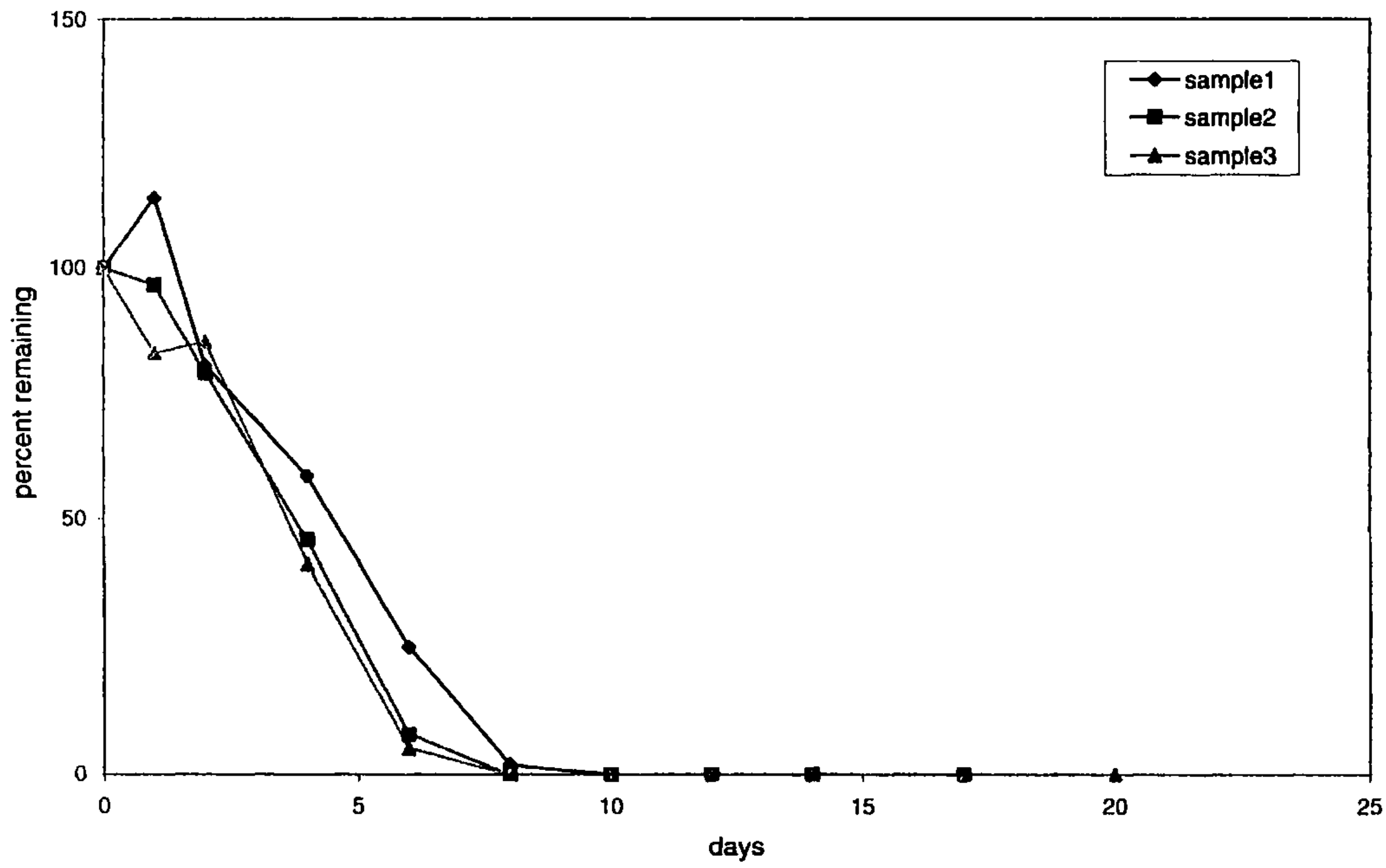
Appendix 27.Percent remaining of S. typhimurium waterborne isolate in Water B, non-filtered , TSA, held at 20C, sample mean, n=2



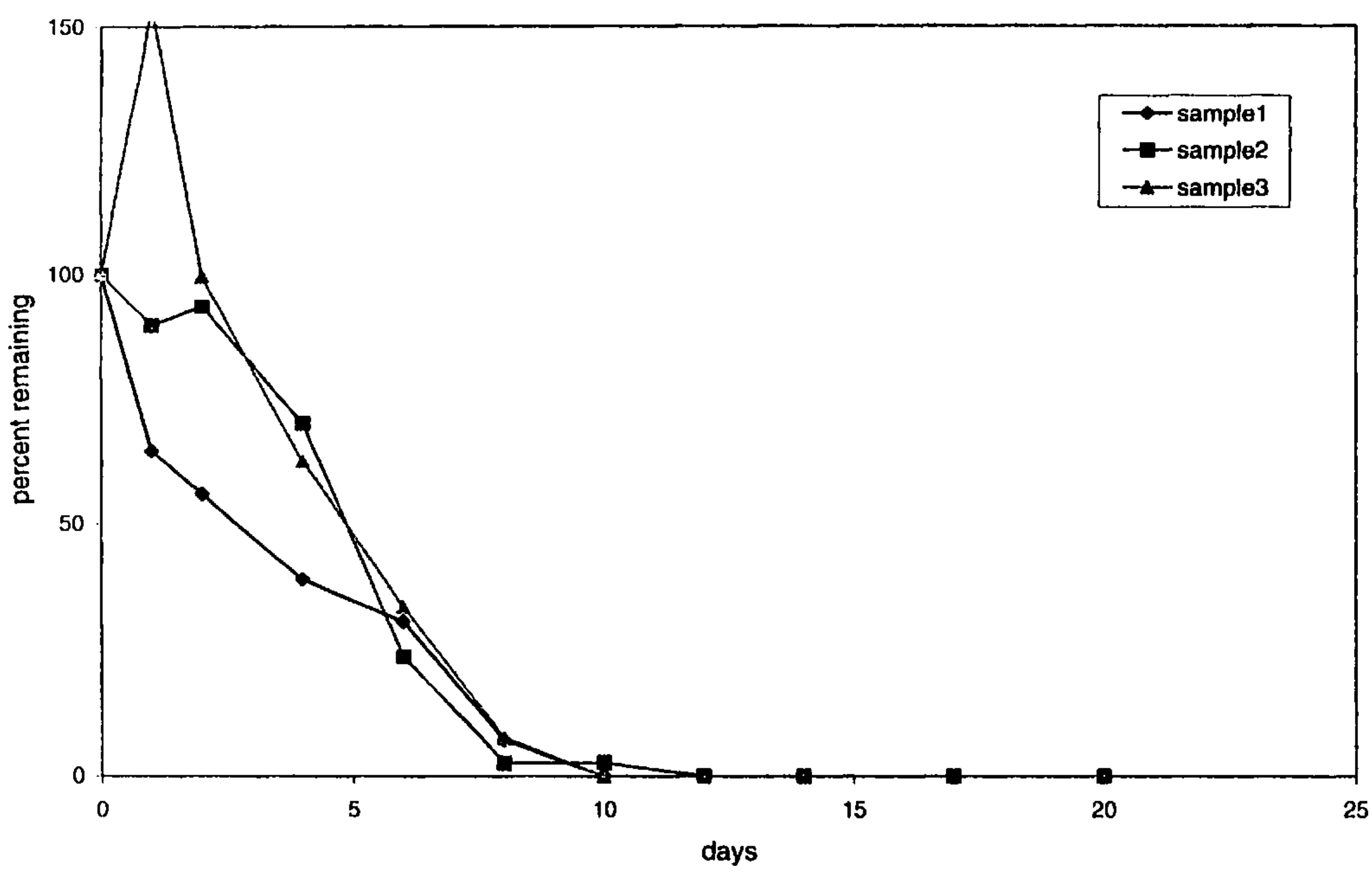
Appendix 28.Percent remaining of E. coliO157:H7 waterborne isolate in
Water B, non-filtered, on TSA, held at 20C,   sample mean, n=2



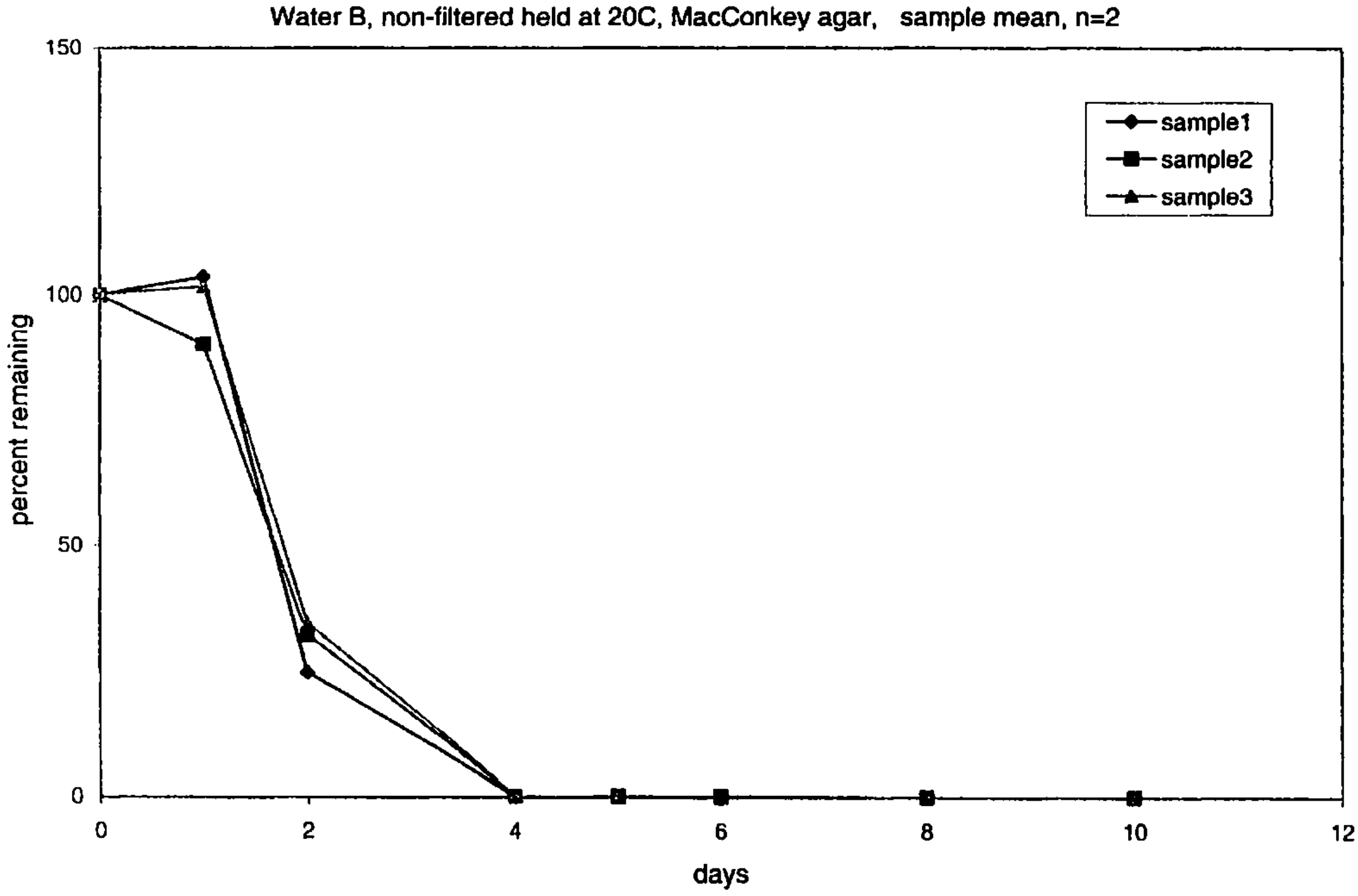
Appendix 29.Percent remaining of S. typhimurium waterborne isolate in Water B, non-filtered held at 10C, MacConkey agar, sample mean, n=2



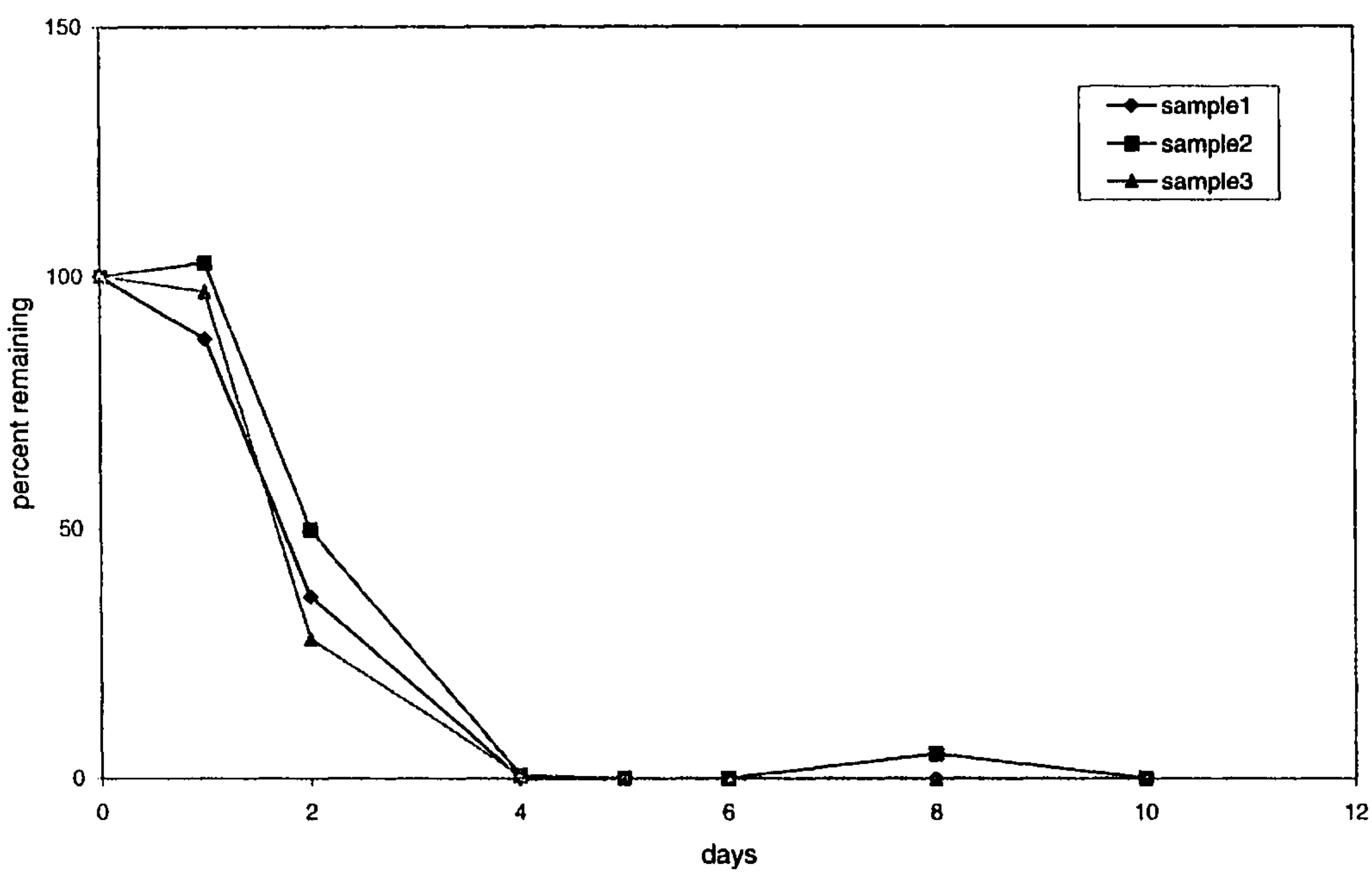
Appendix 30.Percent remaining of E. coliO157:H7 waterborne isolate in
Water B,non-filtered held at 10C, MacConkey agar, sample mean, n=2



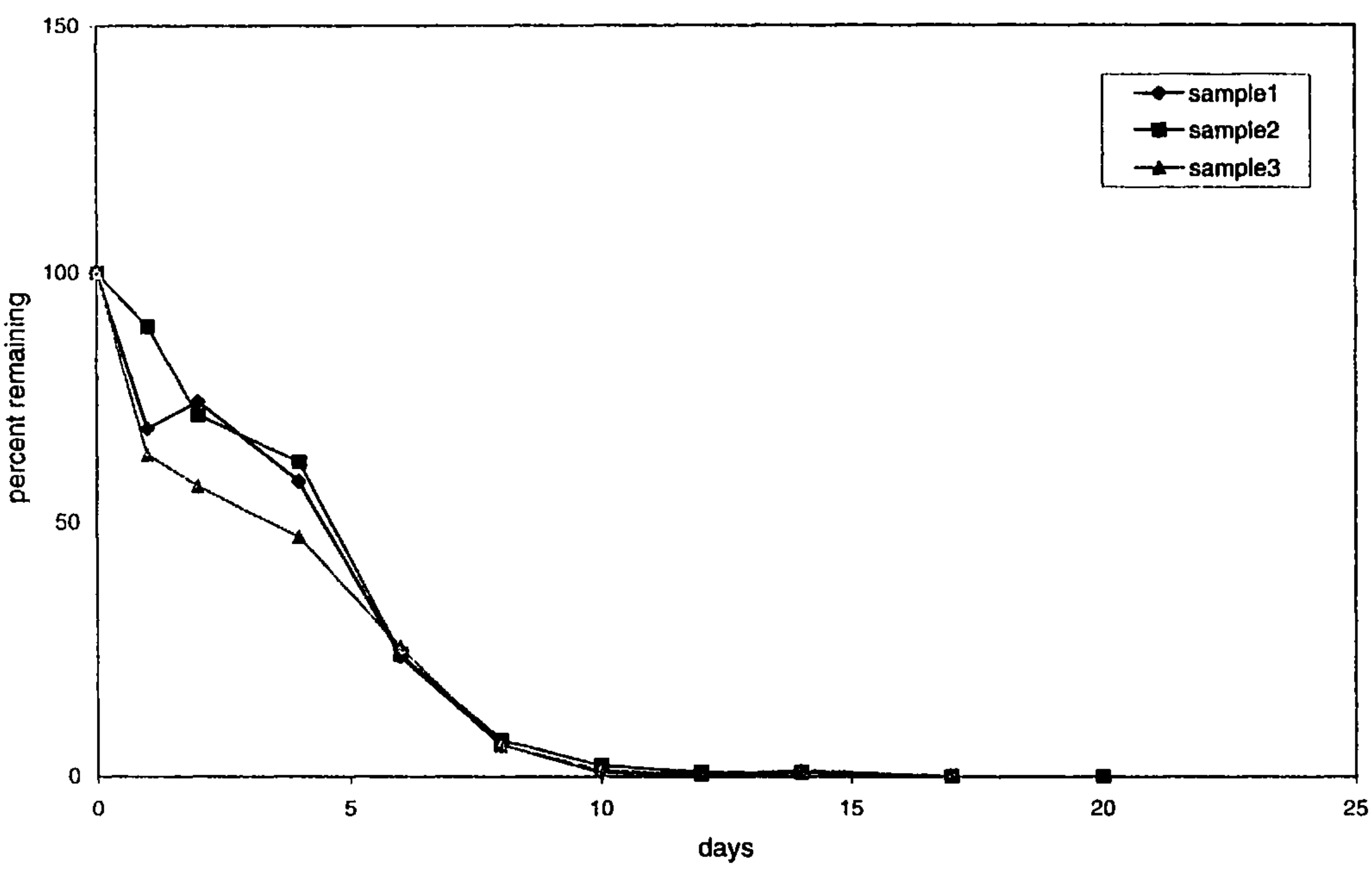
Appendix 31.Percent remaining of S. typhimurium waterborne isolate in Water B, non-filtered held at 20C, MacConkey agar, sample mean, n=2



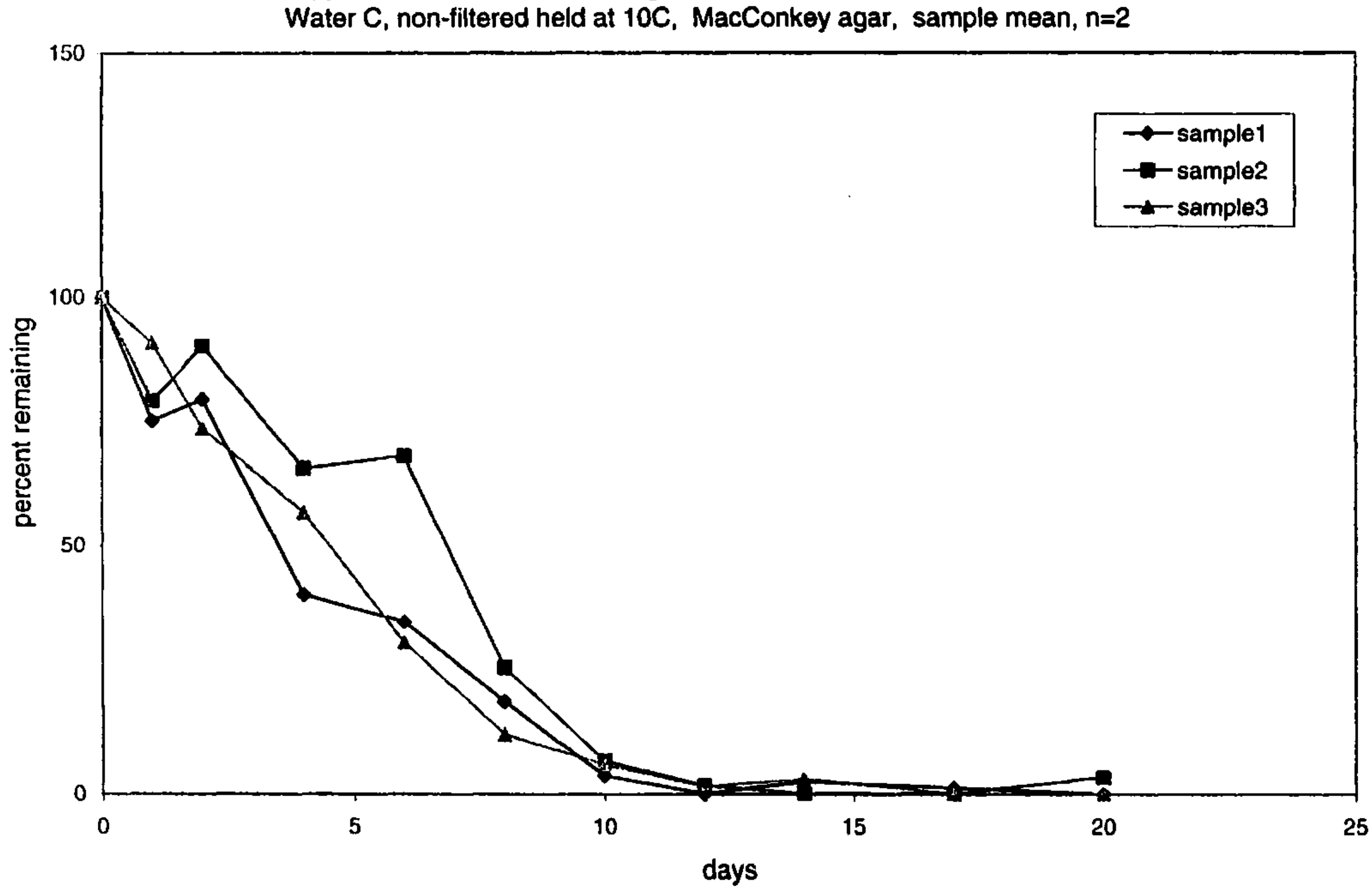
Appendix 32.Percent remaining of E. coliO157:H7 waterborne isolate in Water B, non-filtered held at 20C, MacConkey agar, sample mean, n=2



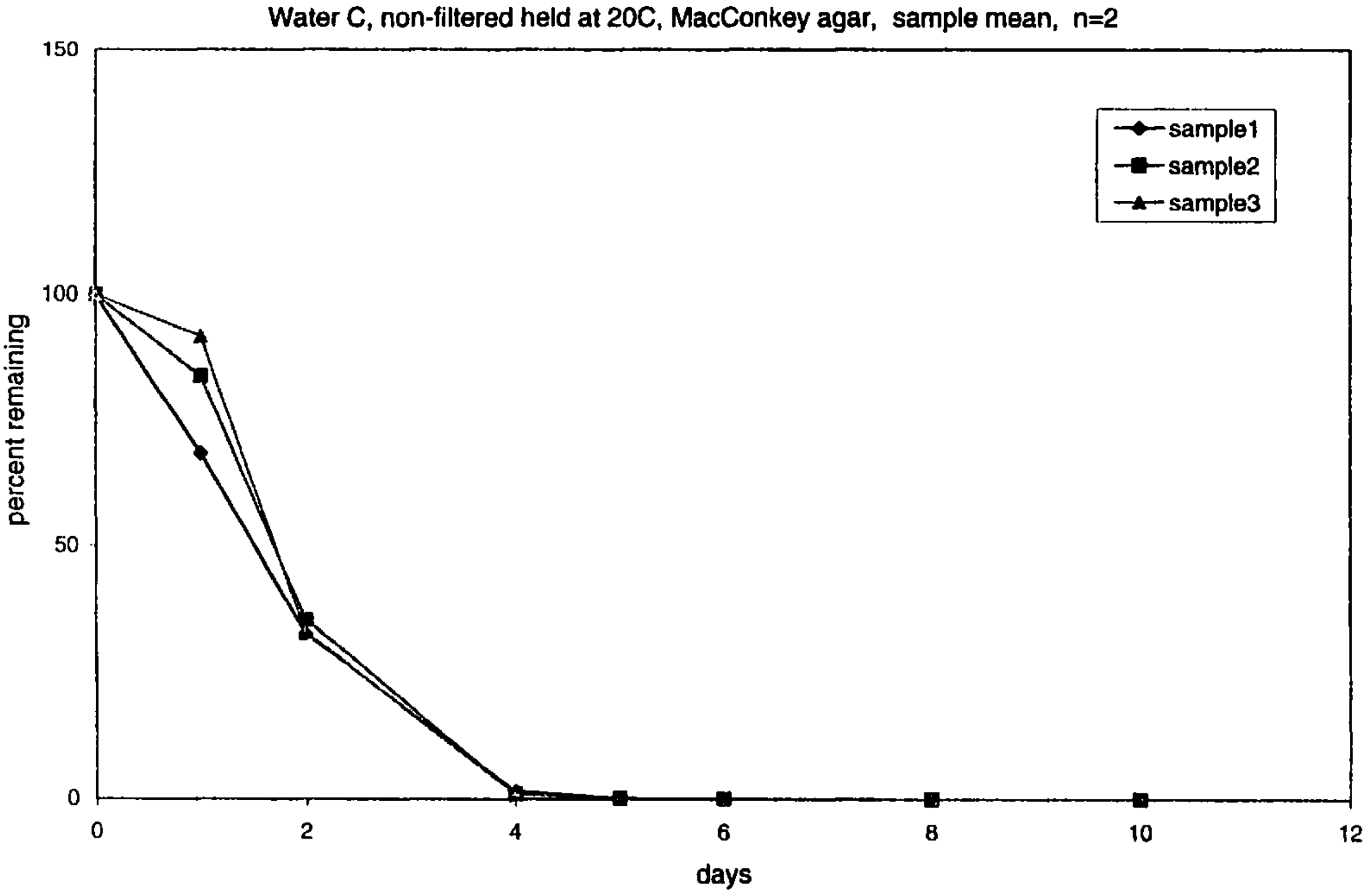
Appendix 33.Percent remaining of S. typhimurium waterborne isolate in Water C, non-filtered held at 10C, MacConkey agar, sample mean, n=2



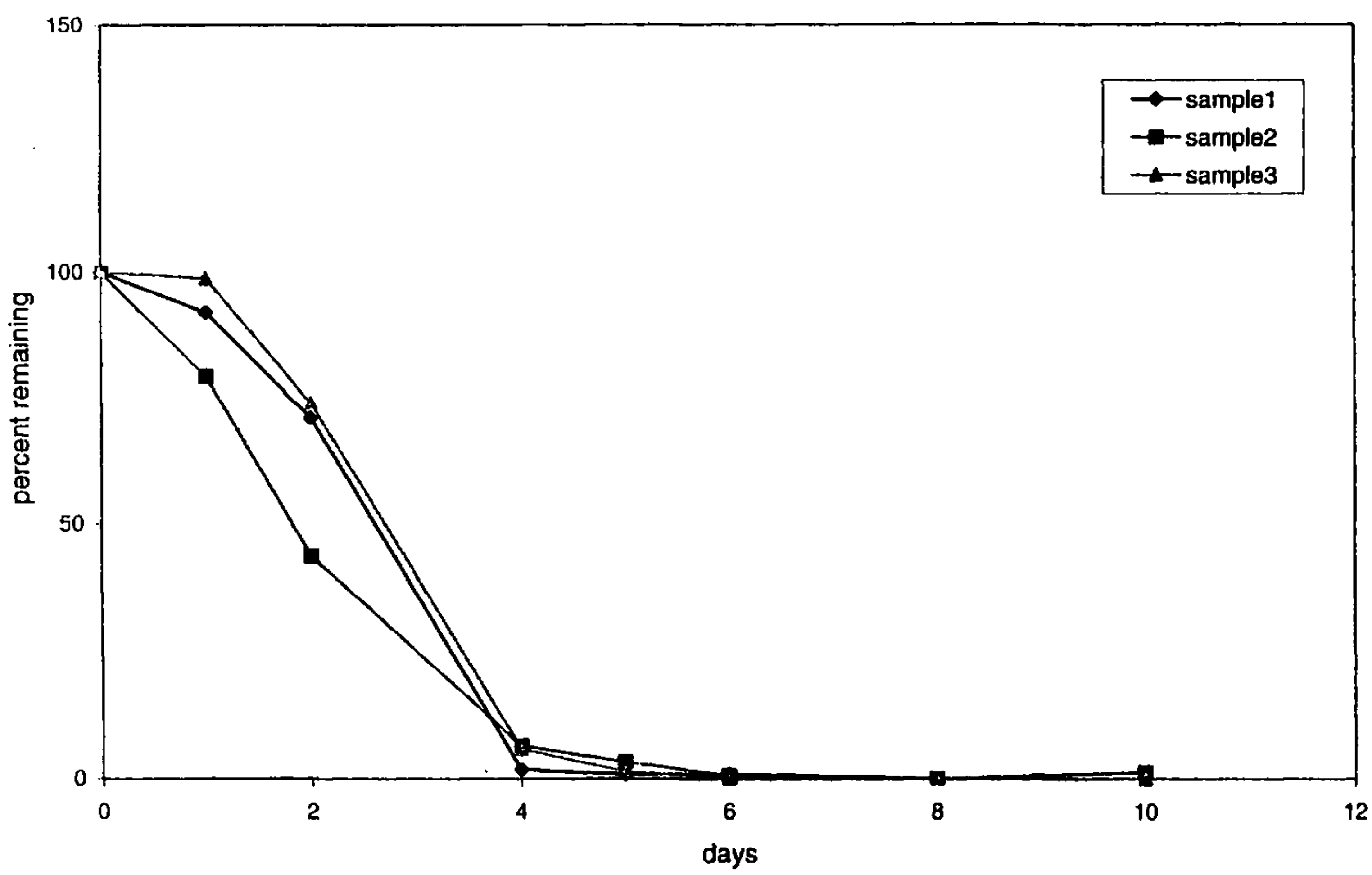
Appendix 34.Percent remaining of E. coliO157:H7 waterborne isolate in Water C, non-filtered held at 10C, MacConkey agar, sample mean, n=2



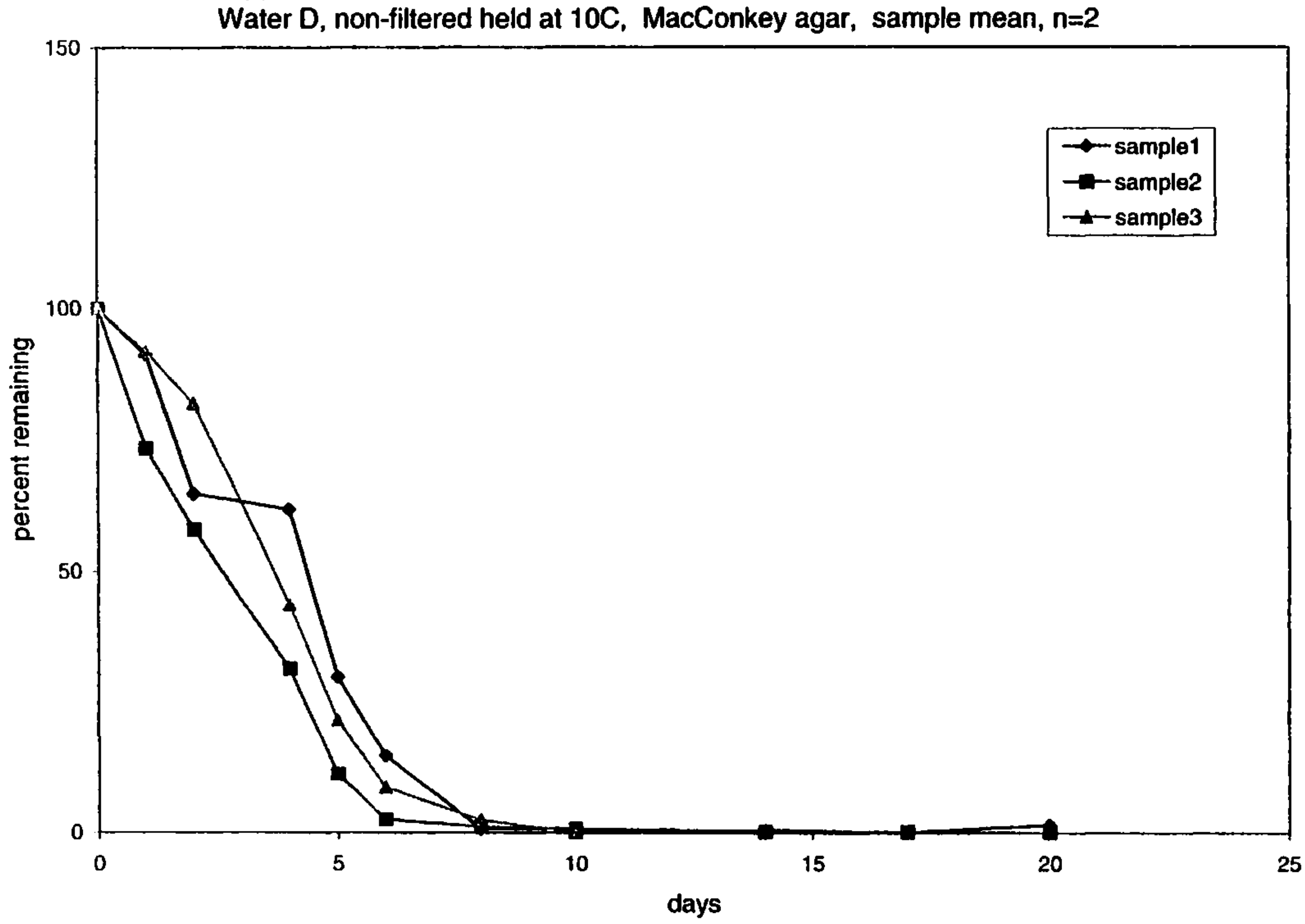
Appendix 35.Percent remaining of S. typhimurium waterborne isolate in Water C, non-filtered held at 20C, MacConkey agar, sample mean, n=2



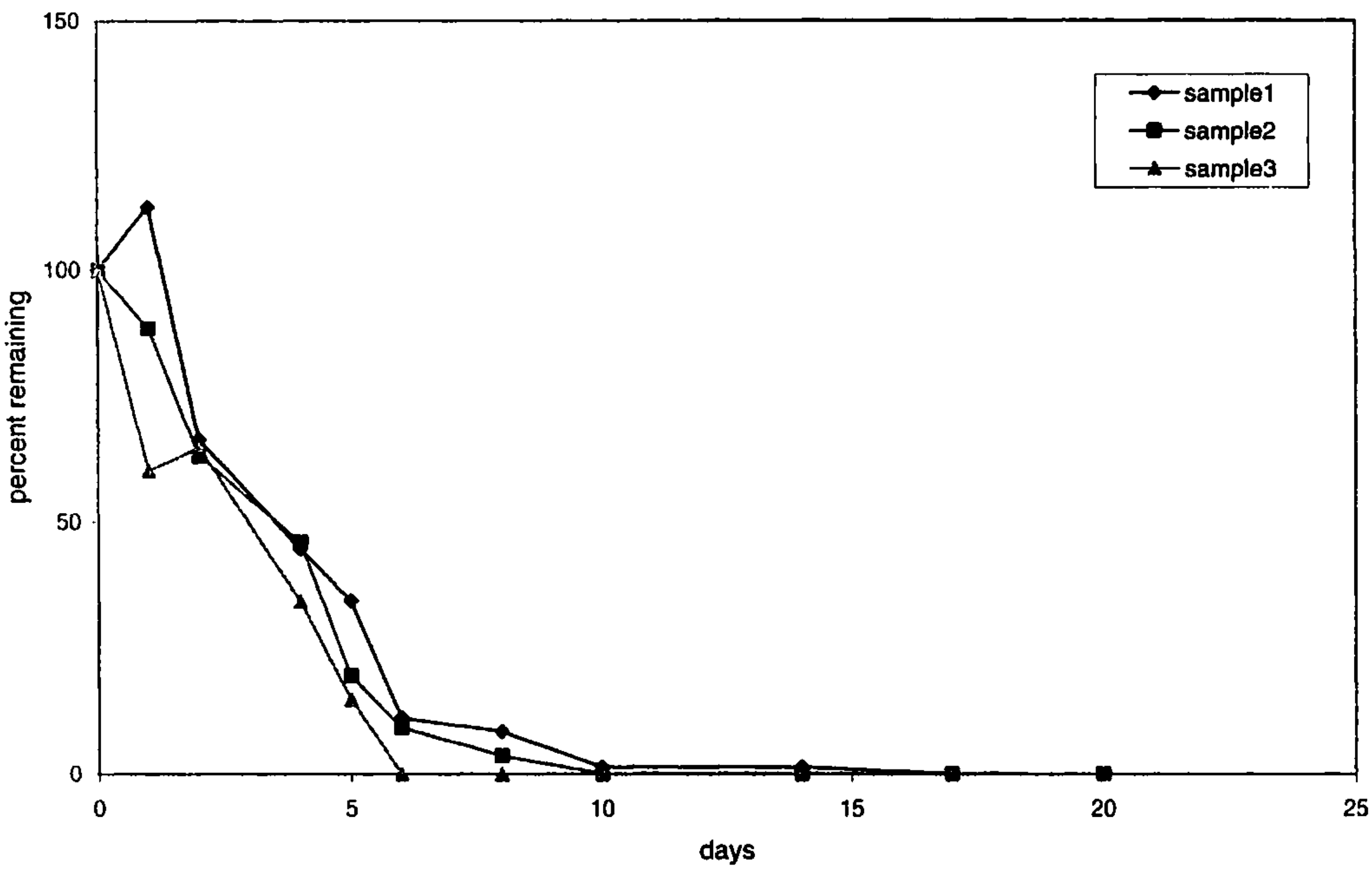
Appendix 36.Percent remaining of E. coliO157:H7 waterborne isolate in Water C, non-filtered held at 20C, MacConkey agar,   sample mean, n=2



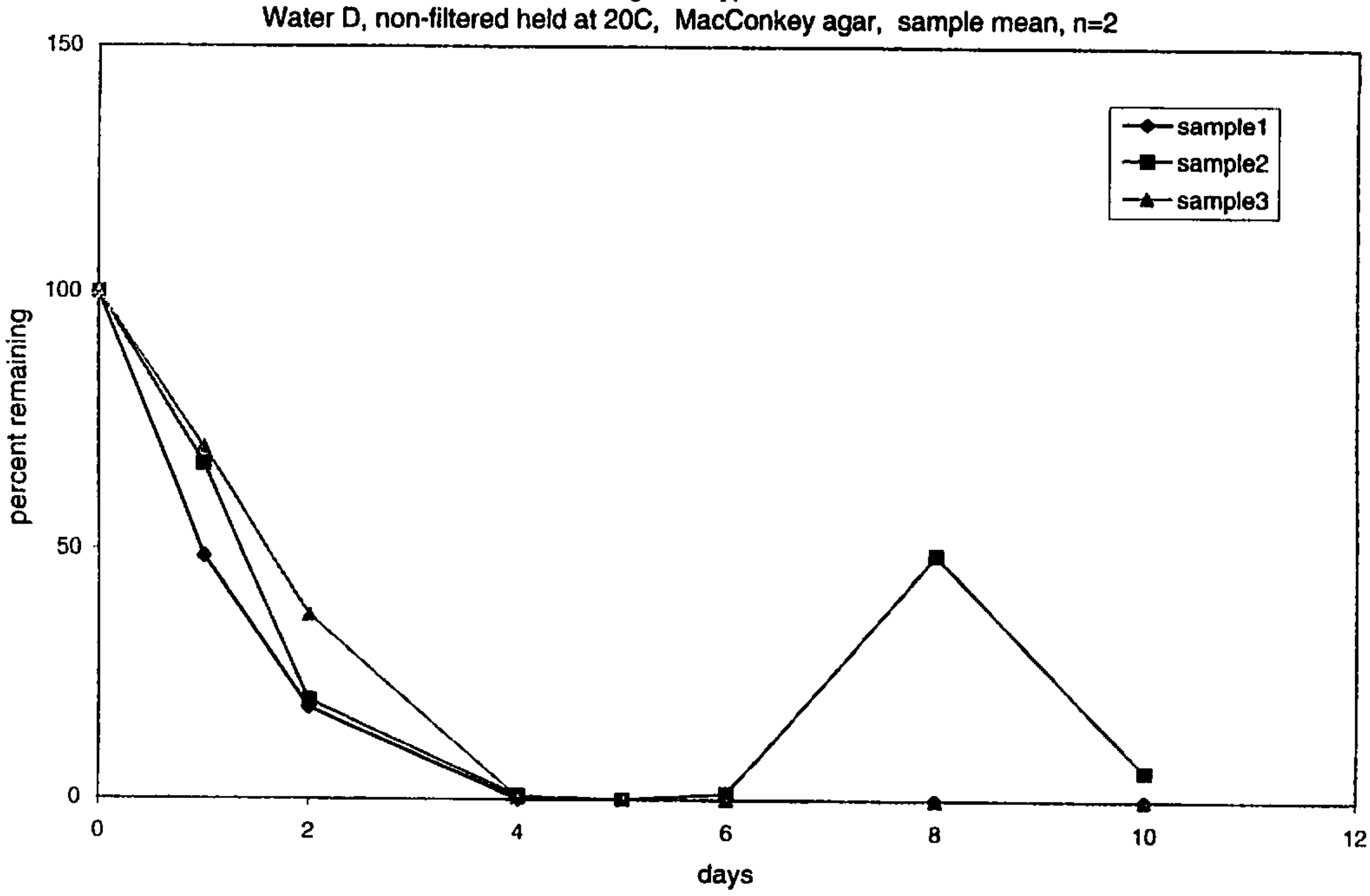
Appendix 37.Percent remaining of S. typhimurium waterborne isolate in Water D, non-filtered held at 10C, MacConkey agar, sample mean, n=2



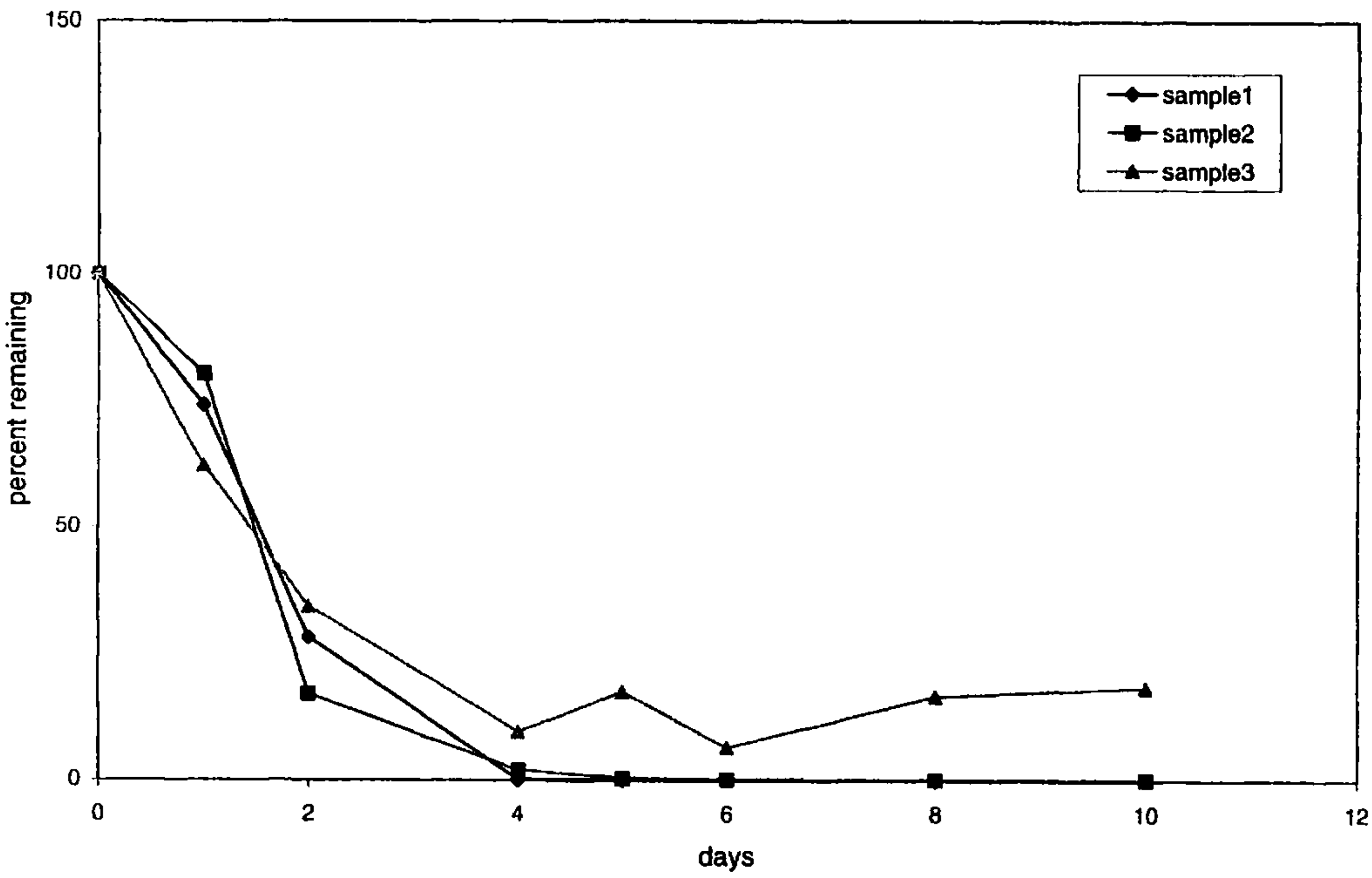
Appendix 38.Percent remaining of E. coliO157:H7 waterborne isolate in Water D, non-filtered held at 10C, MacConkey agar, sample mean, n=2



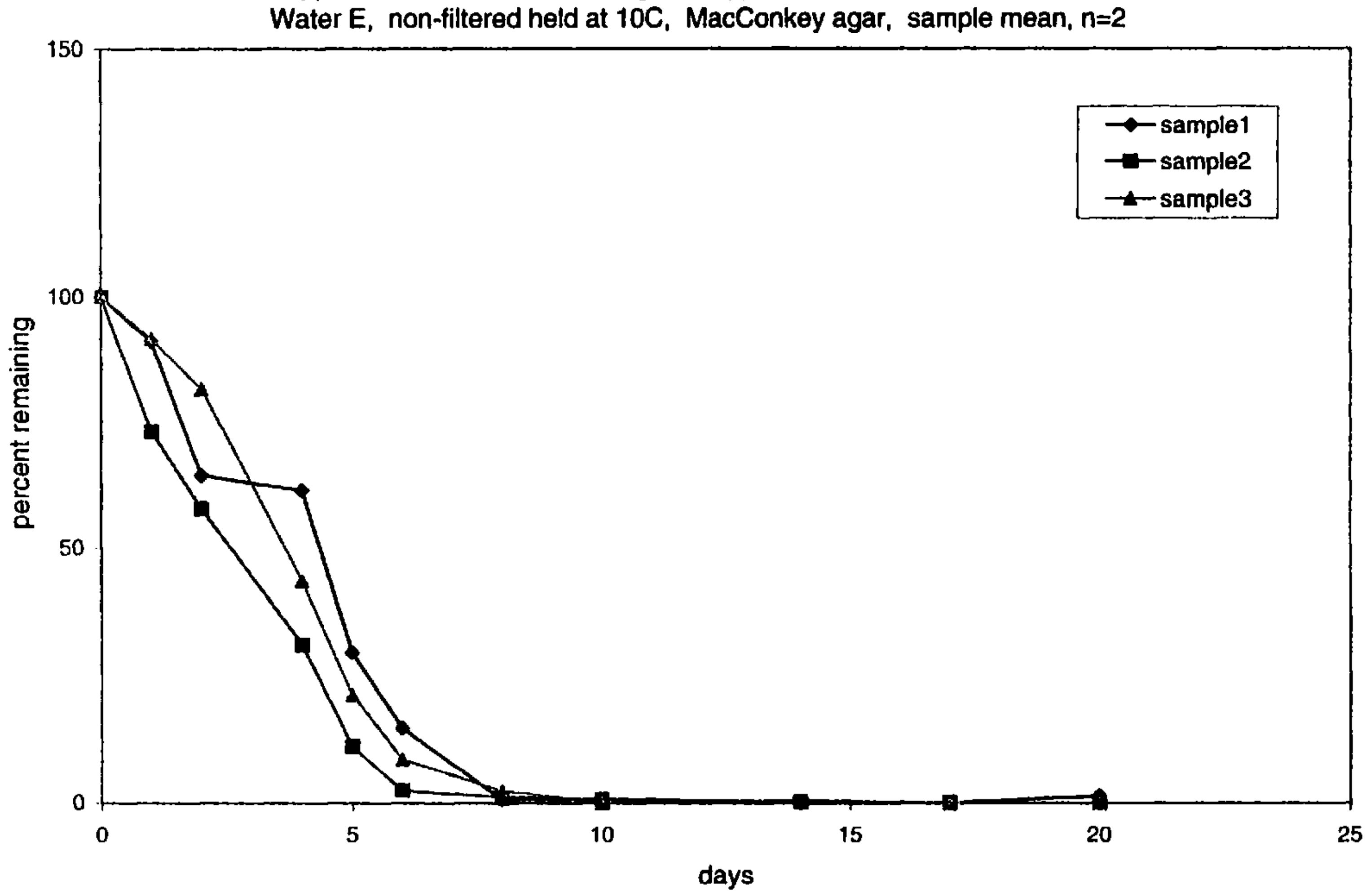
Appendix 39.Percent remaining of S. typhimurium waterborne isolate in Water D, non-filtered held at 20C, MacConkey agar, sample mean, n=2



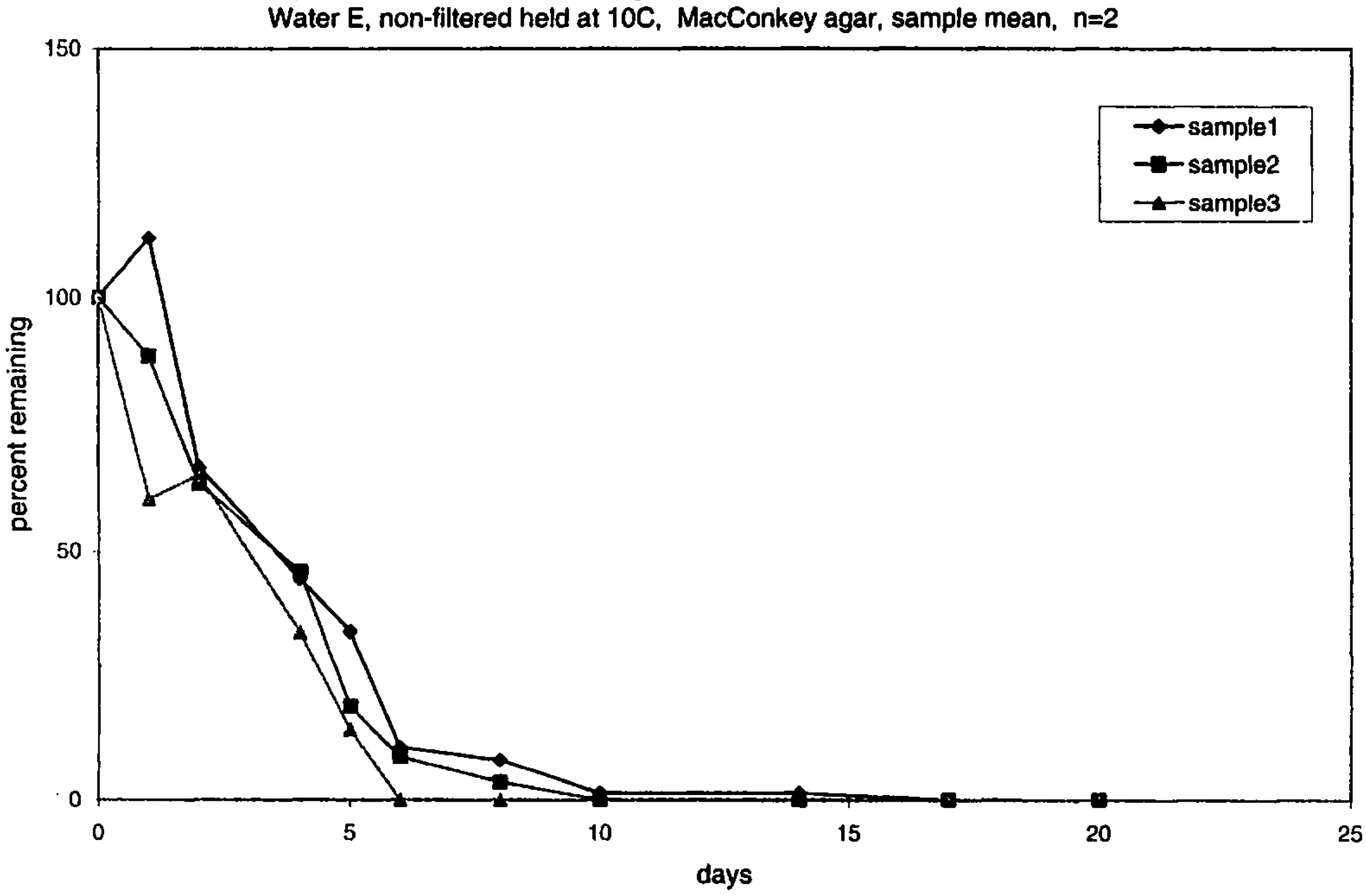
Appendix 40.Percent remaining of E. coliO157:H7 waterborne isolate in Water D, non-filtered held at 20C, MacConkey agar, sample mean, n=2



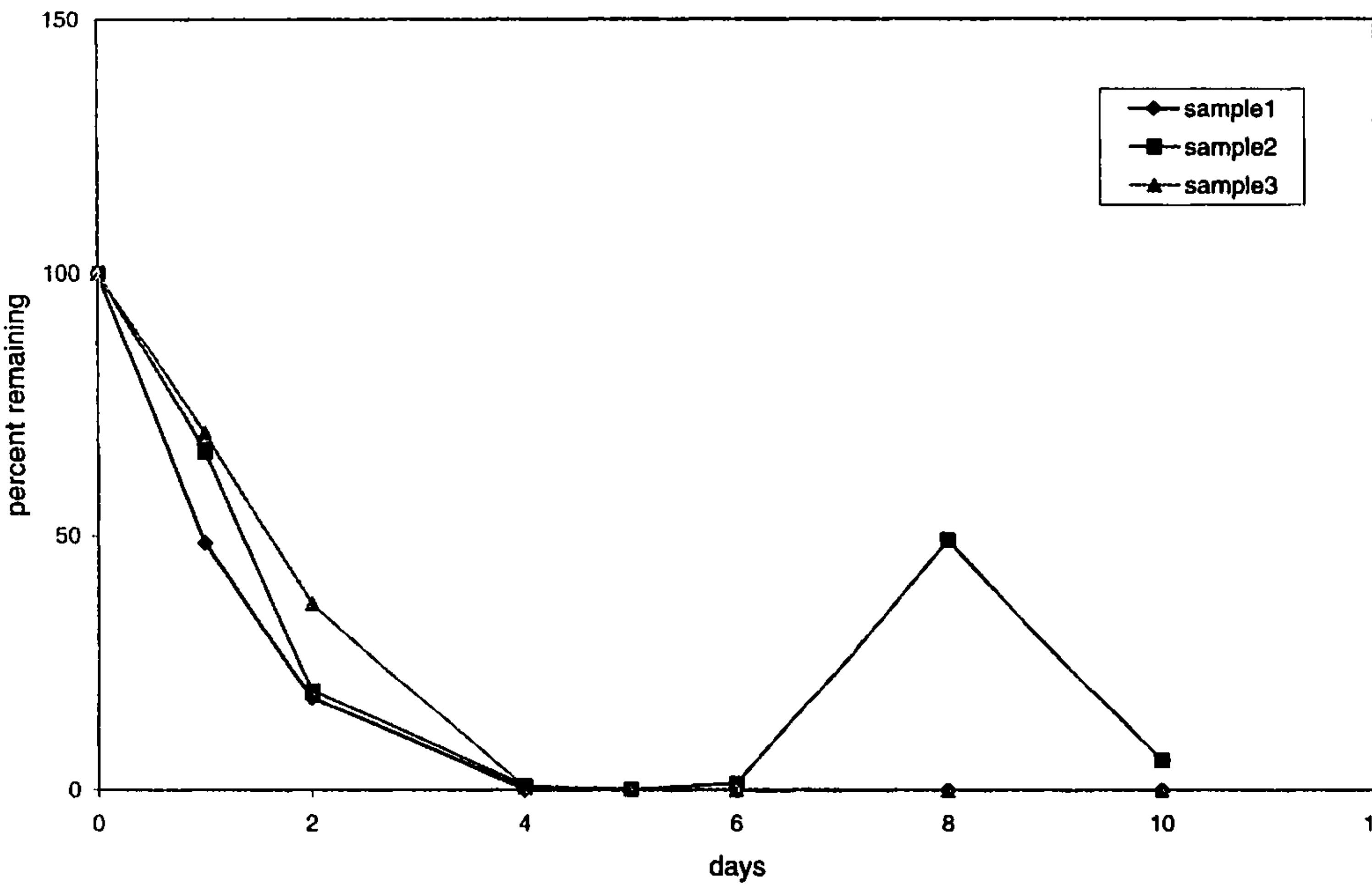
Appendix 41.Percent remaining of S. typhimurium waterborne isolate in Water E, non-filtered held at 10C, MacConkey agar, sample mean, n=2



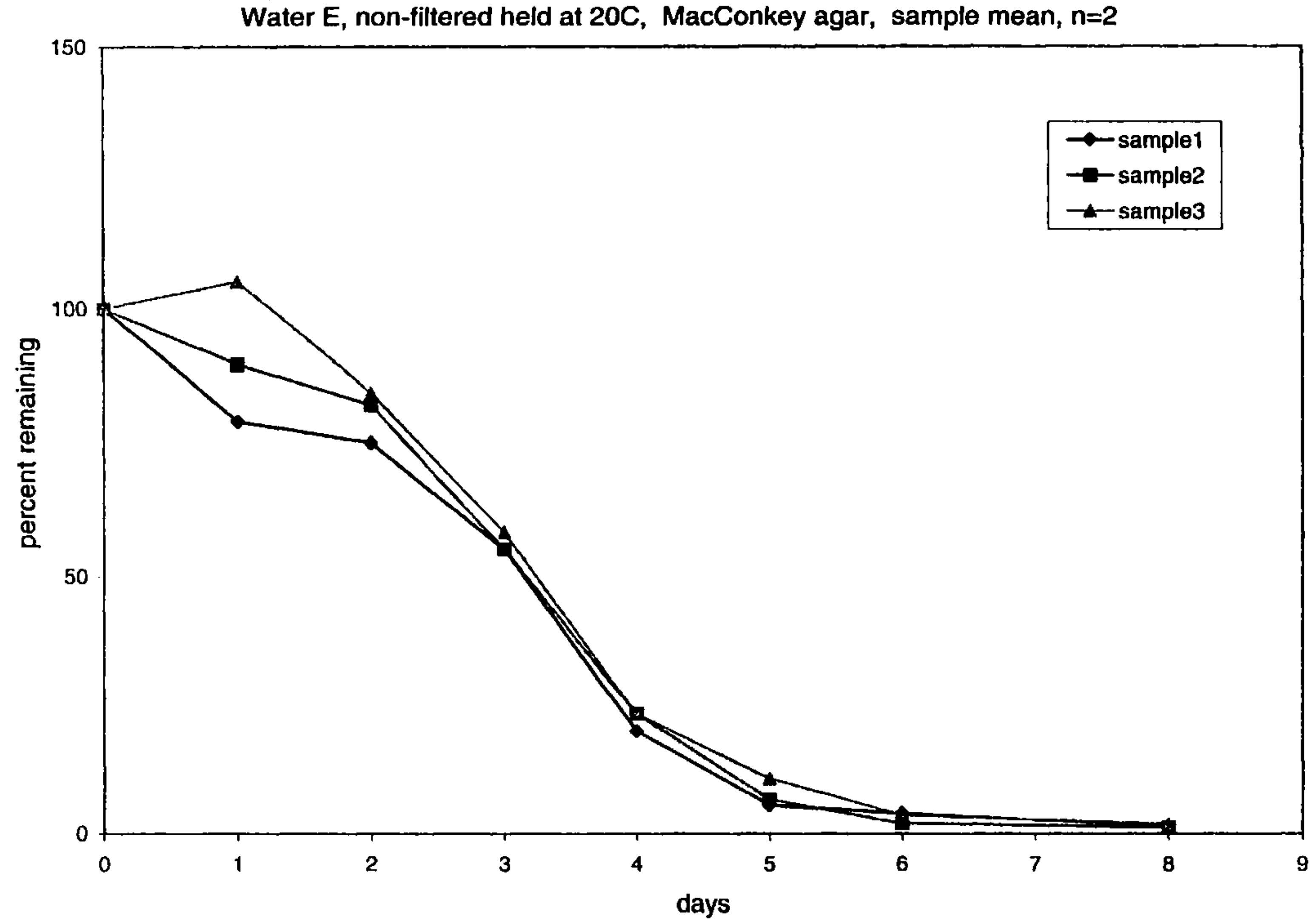
Appendix 42.Percent remaining of E. coliO157:H7 waterborne isolate in Water E, non-filtered held at 10C, MacConkey agar, sample mean, n=2



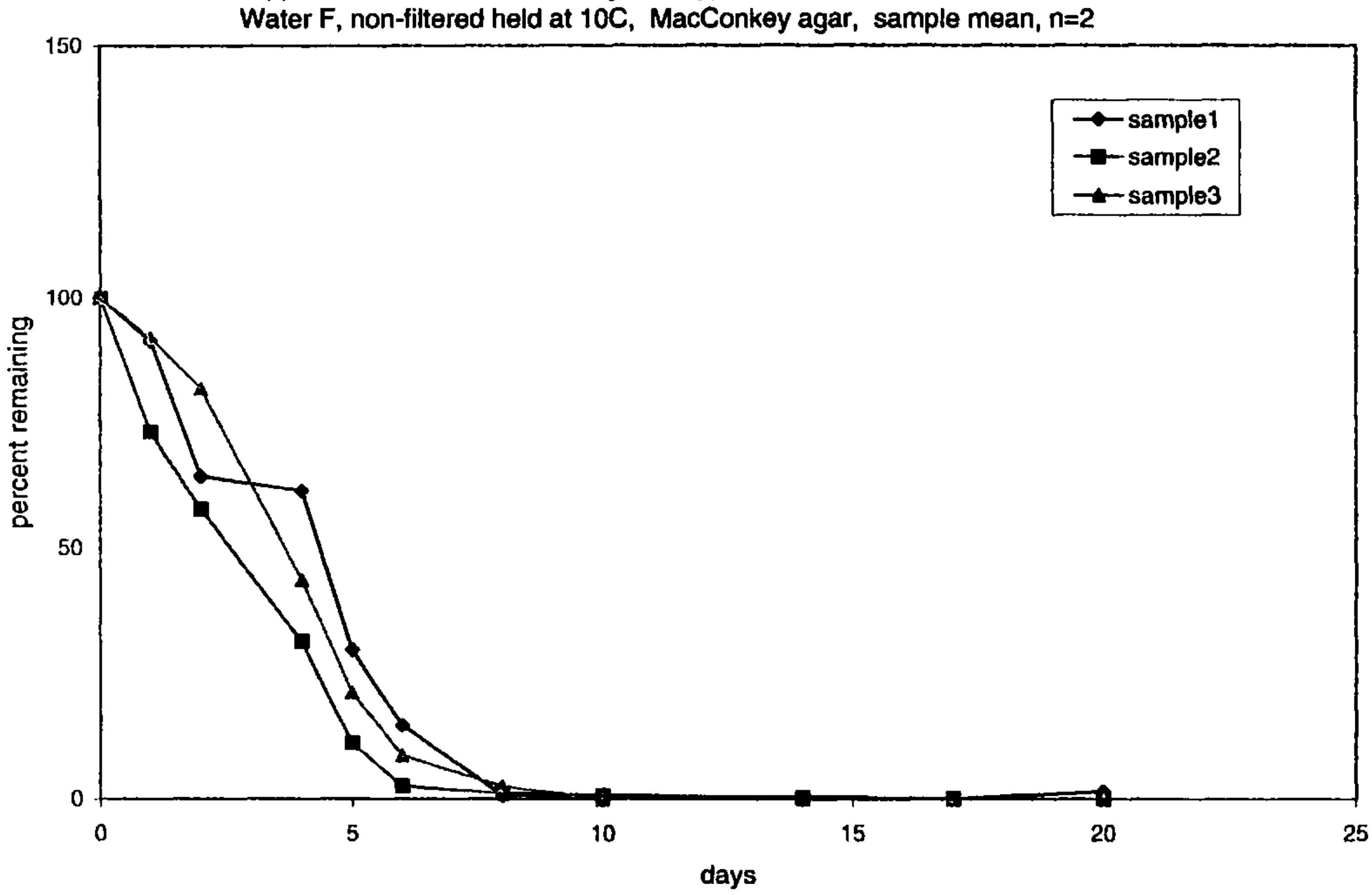
Appendix 43.Percent remaining of S. typhimurium waterborne isolate in Water E, non-filtered held at 20C, MacConkey agar, sample mean, n=2

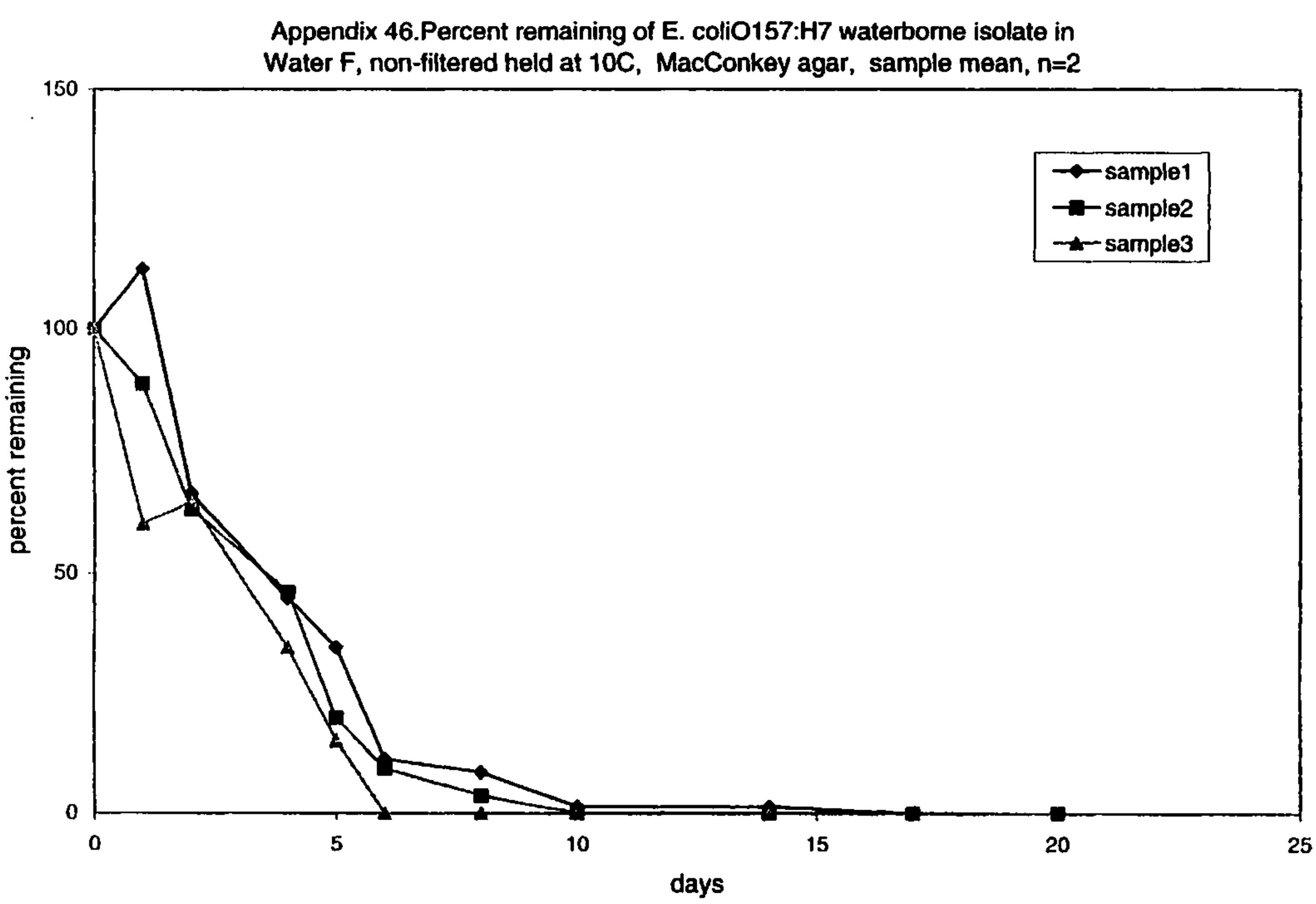


Appendix 44.Percent remaining of E. coliO157:H7 waterborne isolate in Water E, non-filtered held at 20C, MacConkey agar, sample mean, n=2

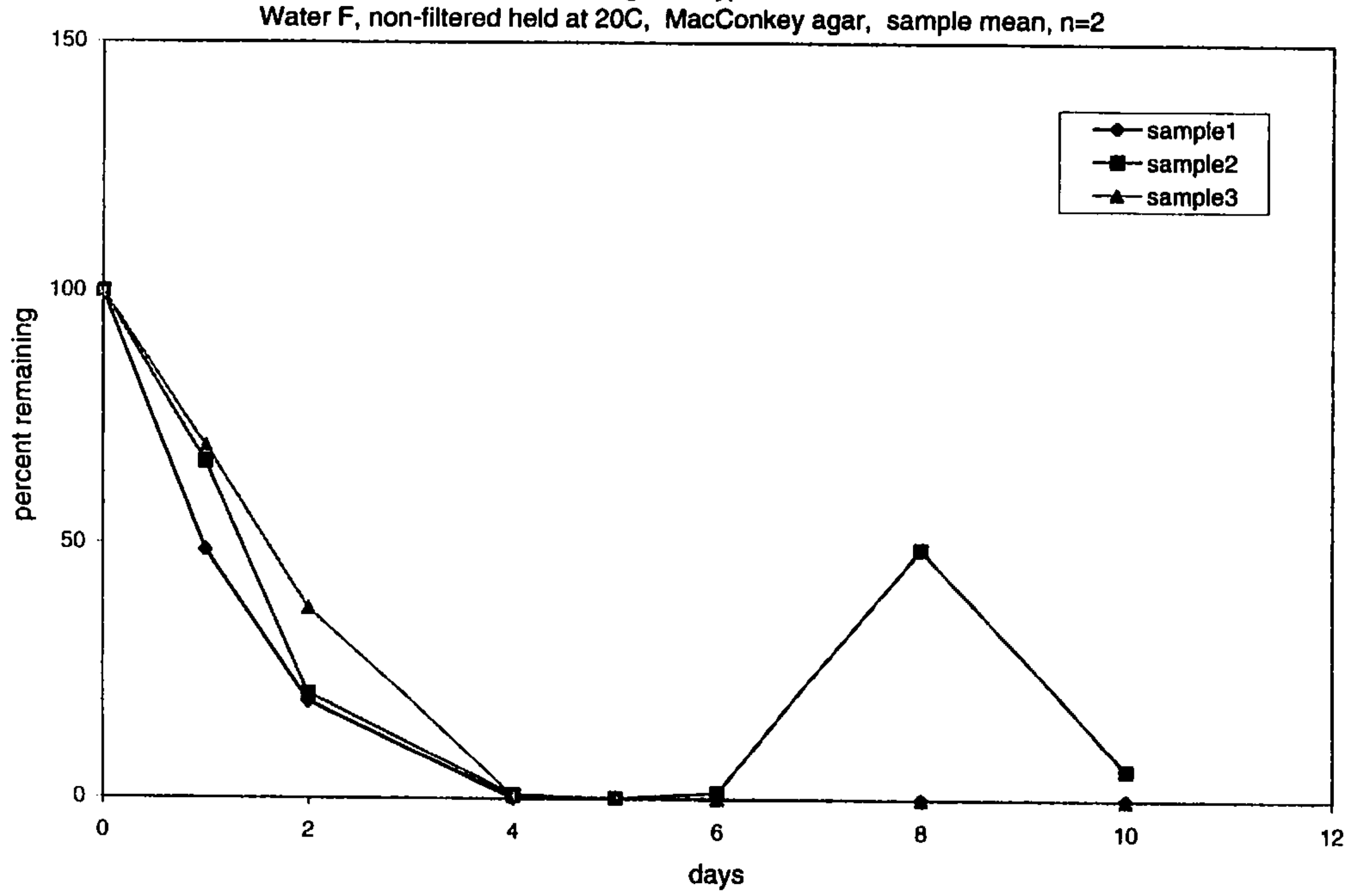


Appendix 45.Percent remaining of S. typhimurium waterborne isolate in Water F, non-filtered held at 10C, MacConkey agar, sample mean, n=2

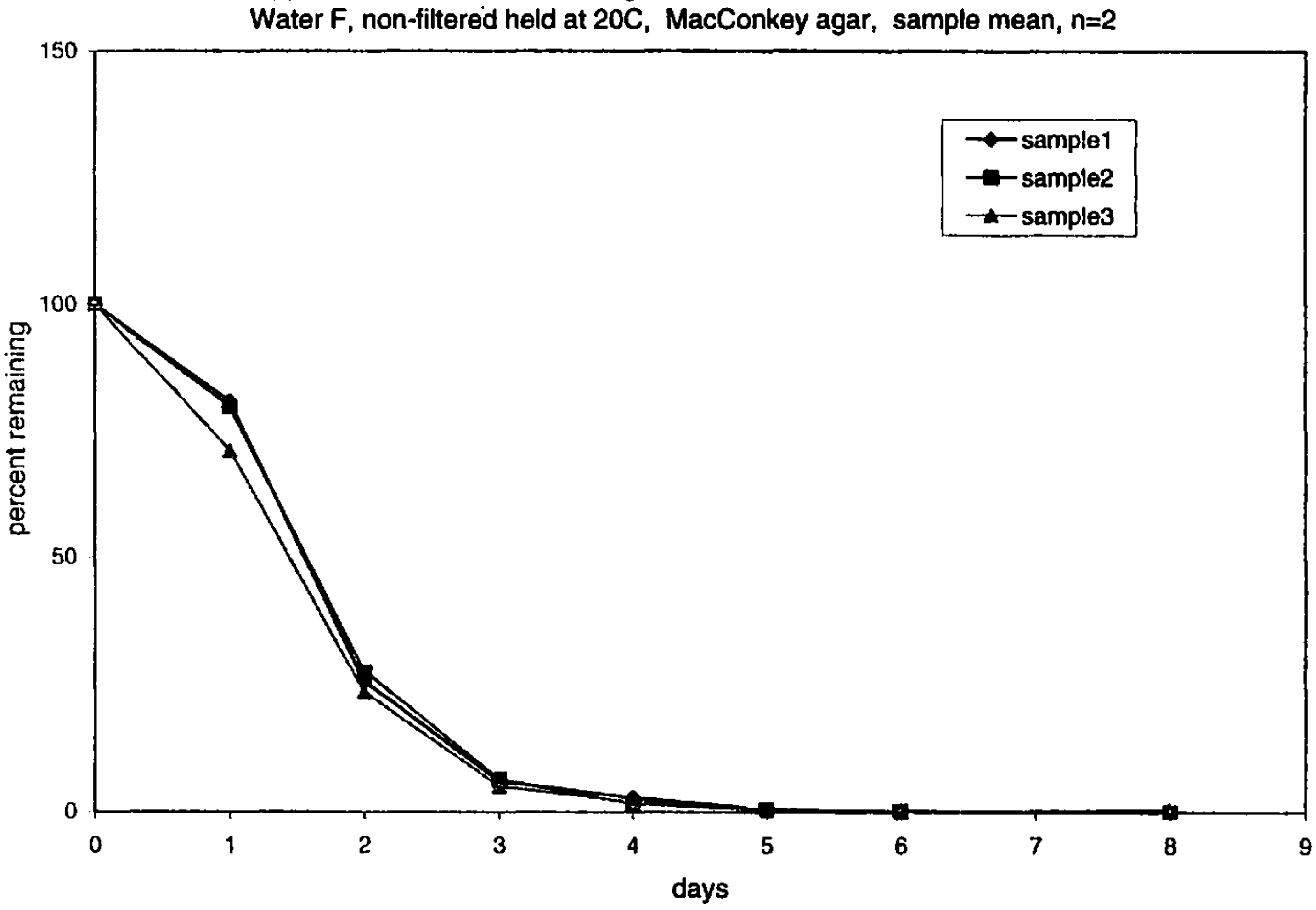



Appendix 46.Percent remaining of E. coliO157:H7 waterborne isolate in
Water F, non-filtered held at 10C, MacConkey agar, sample mean, n=2
150
100
50
0
percent remaining
0
5
10
15
20
25
days
sample1
sample2
sample3

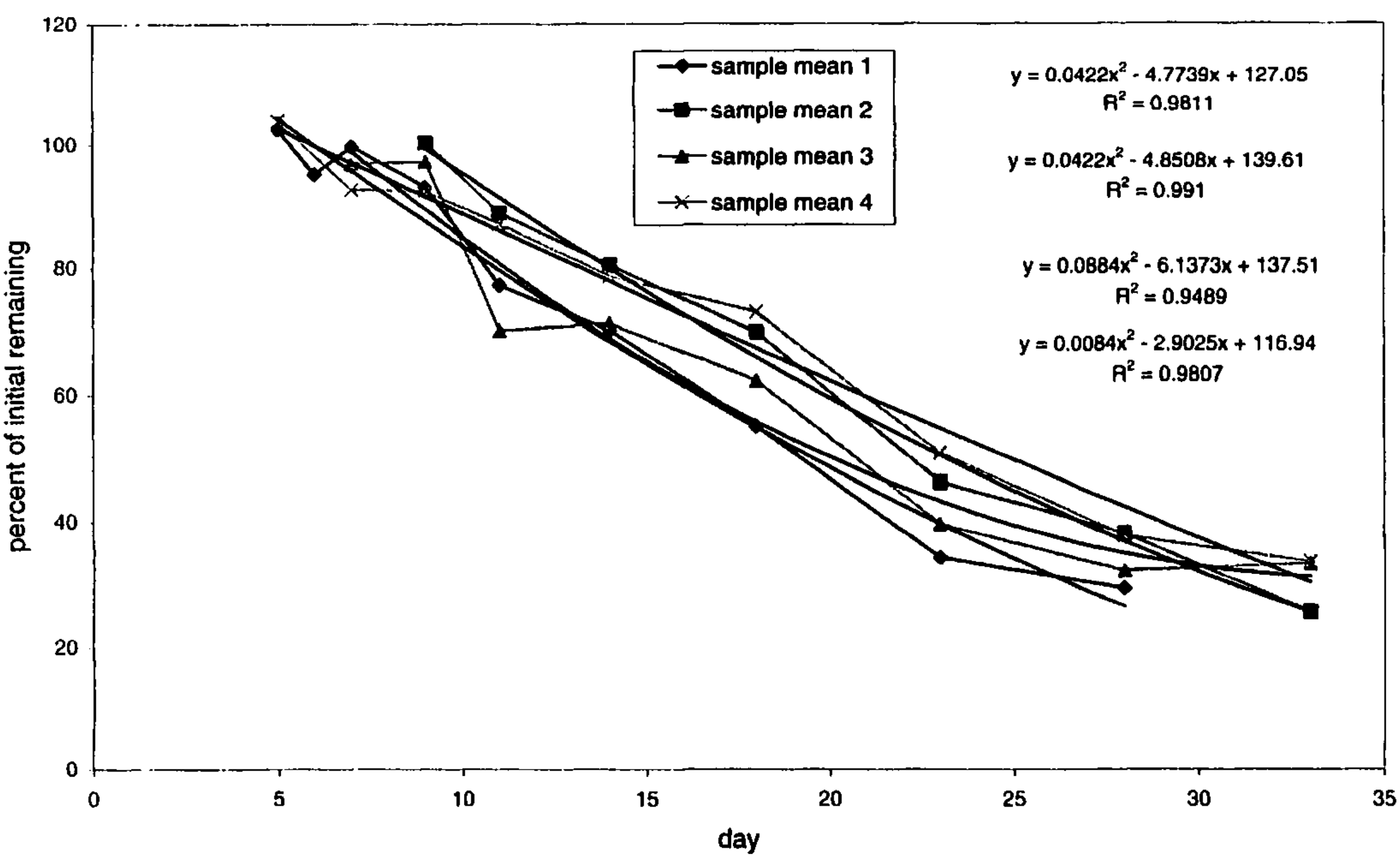
Appendix 47. Percent remaining of S. typhimurium waterborne isolate in Water F, non-filtered held at 20C, MacConkey agar, sample mean, n=2



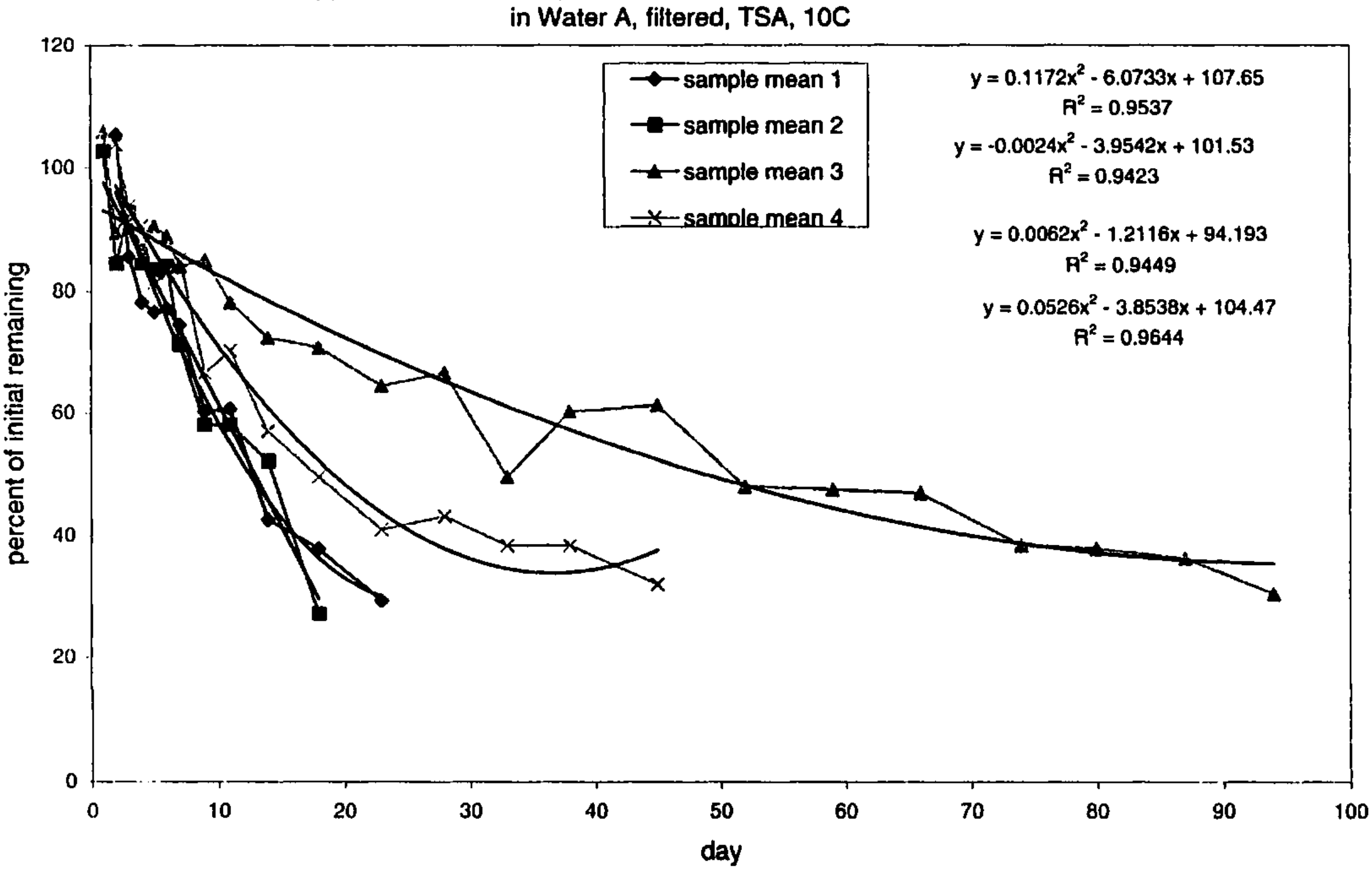
Appendix 48. Percent remaining of E. coliO157:H7 waterborne isolate in Water F, non-filtered held at 20C, MacConkey agar, sample mean, n=2



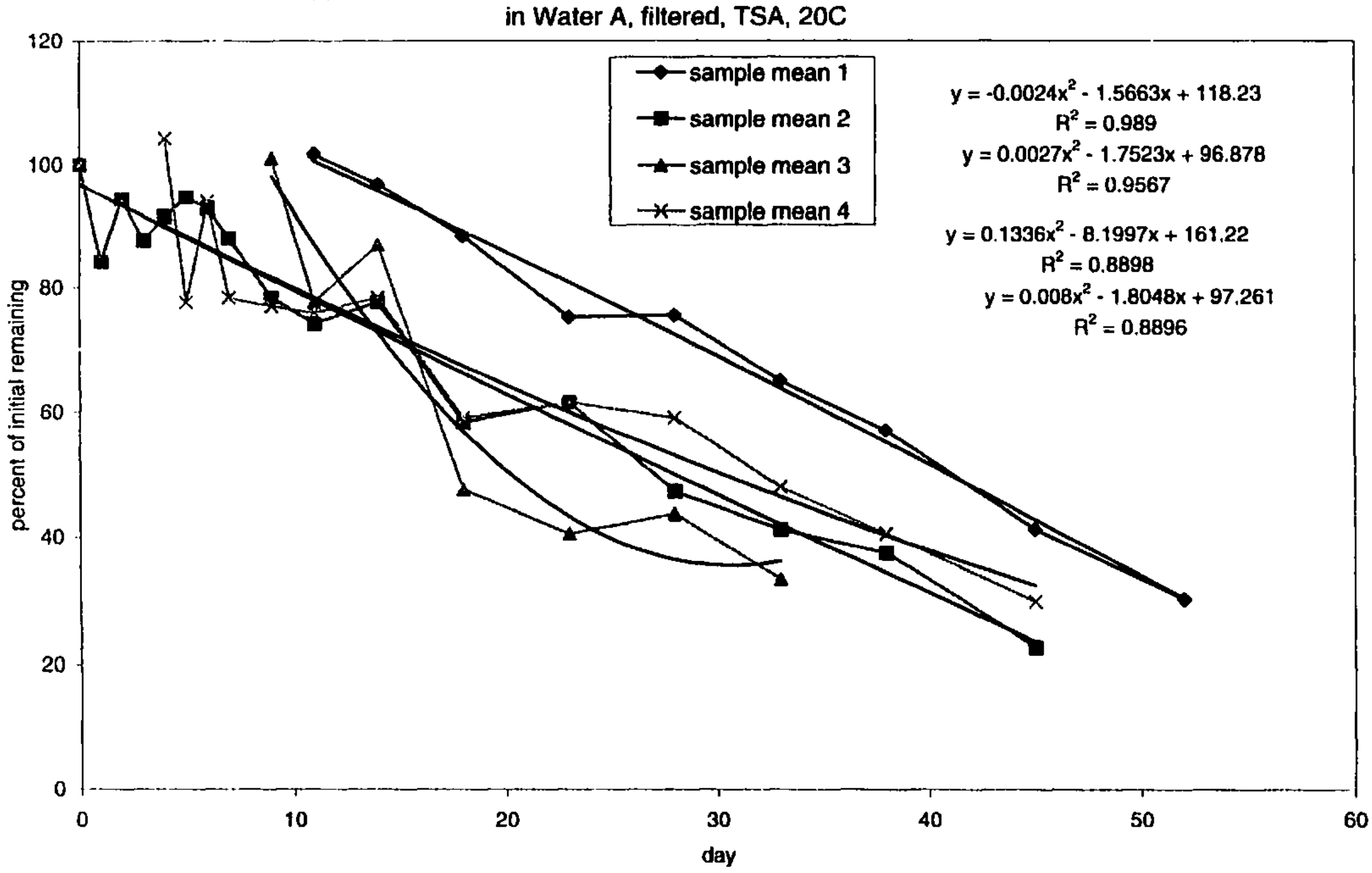
Appendix 49. Decline phase of S. typhimurium waterborne isolate, in Water A filtered, TSA, 10C



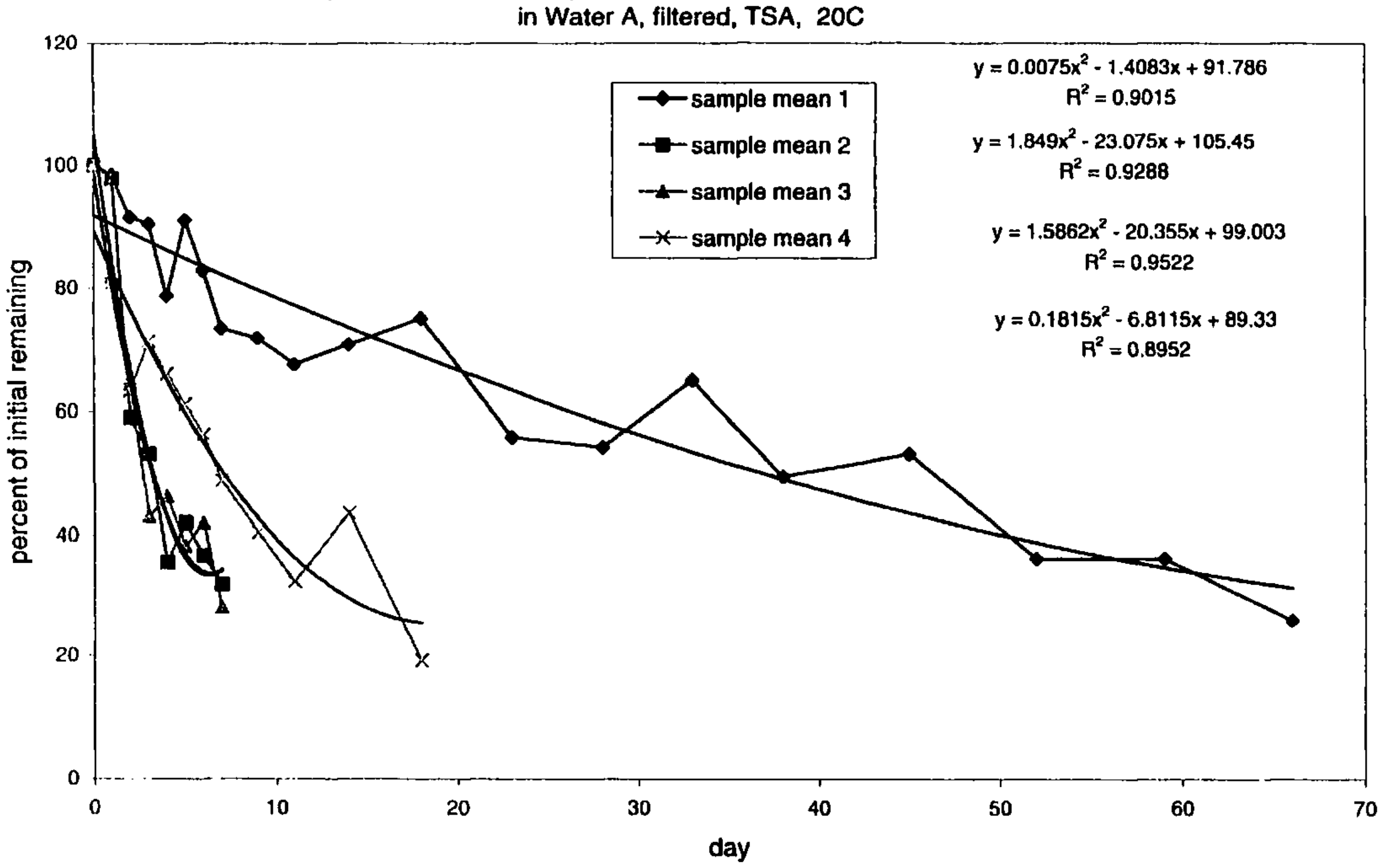
Appendix 50. Decline phase of E. coliO157:H7 waterborne isolate, in Water A, filtered, TSA, 10C



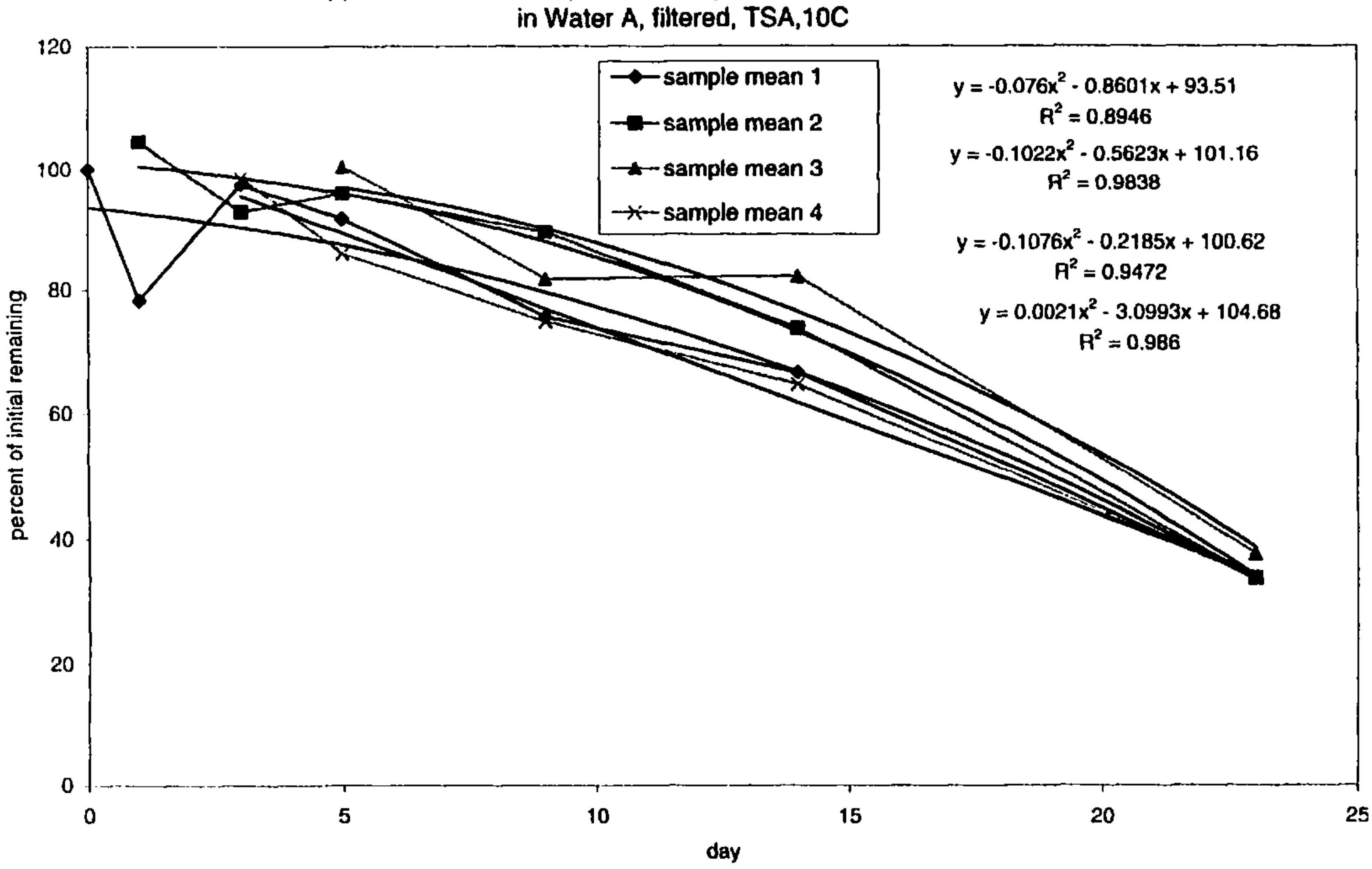
Appendix 51. Decline phase of S. typhimurium waterborne isolate, in Water A, filtered, TSA, 20C



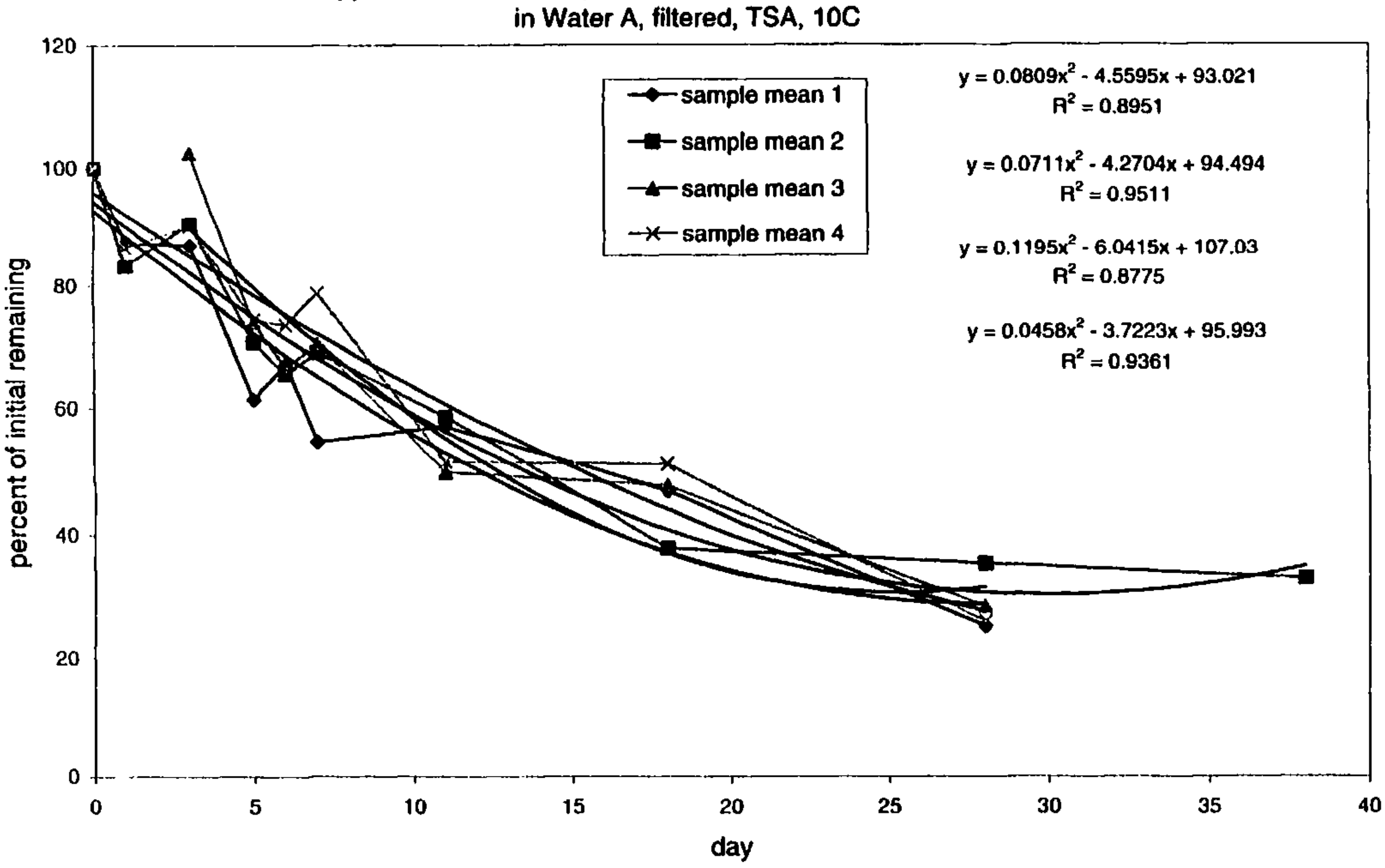
Appendix 52. Decline phase of E. coliO157:H7 waterborne isolate, in Water A, filtered, TSA, 20C



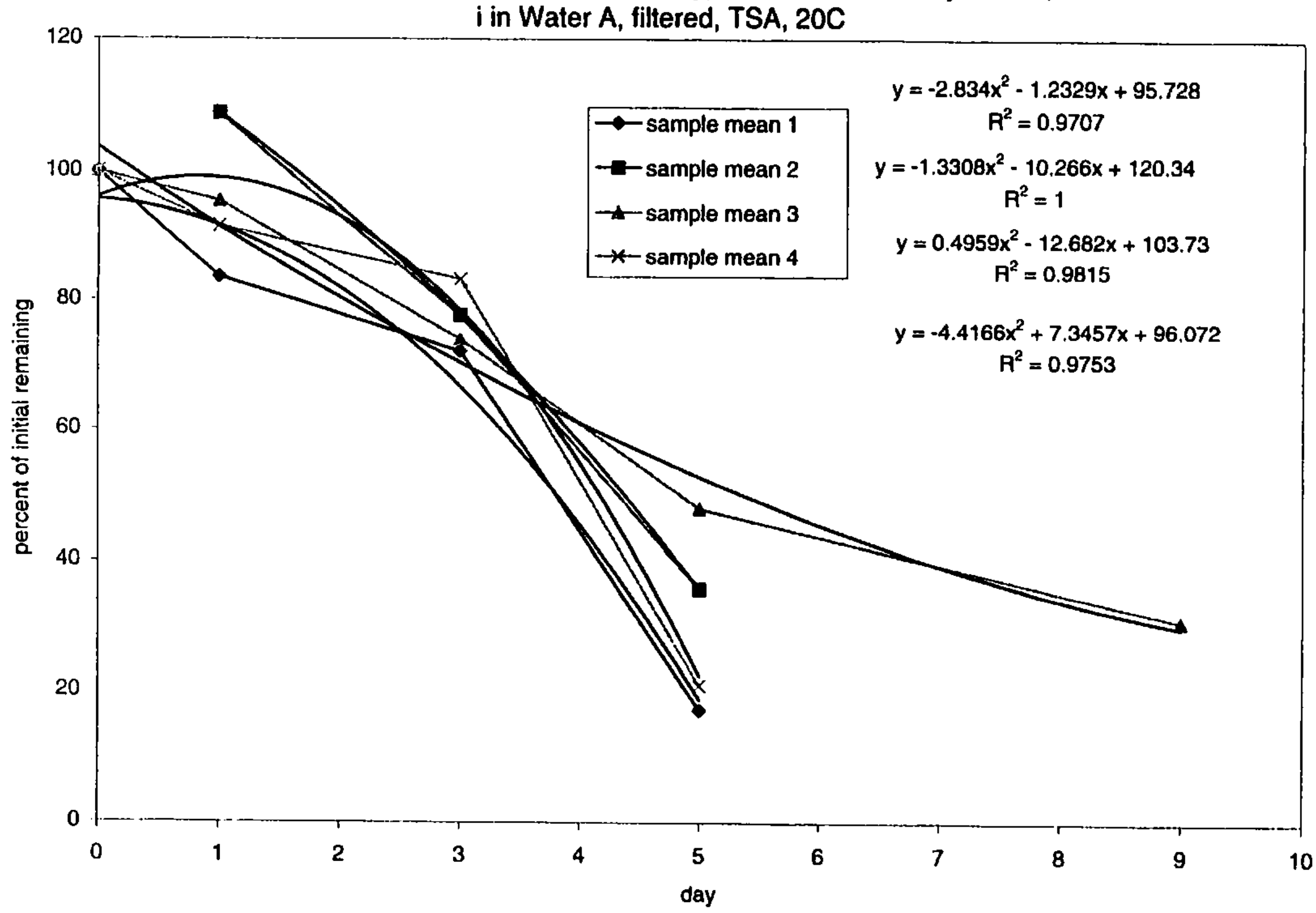
Appendix 53. Decline phase of S. typhimurium laboratory isolate, in Water A, filtered, TSA,10C



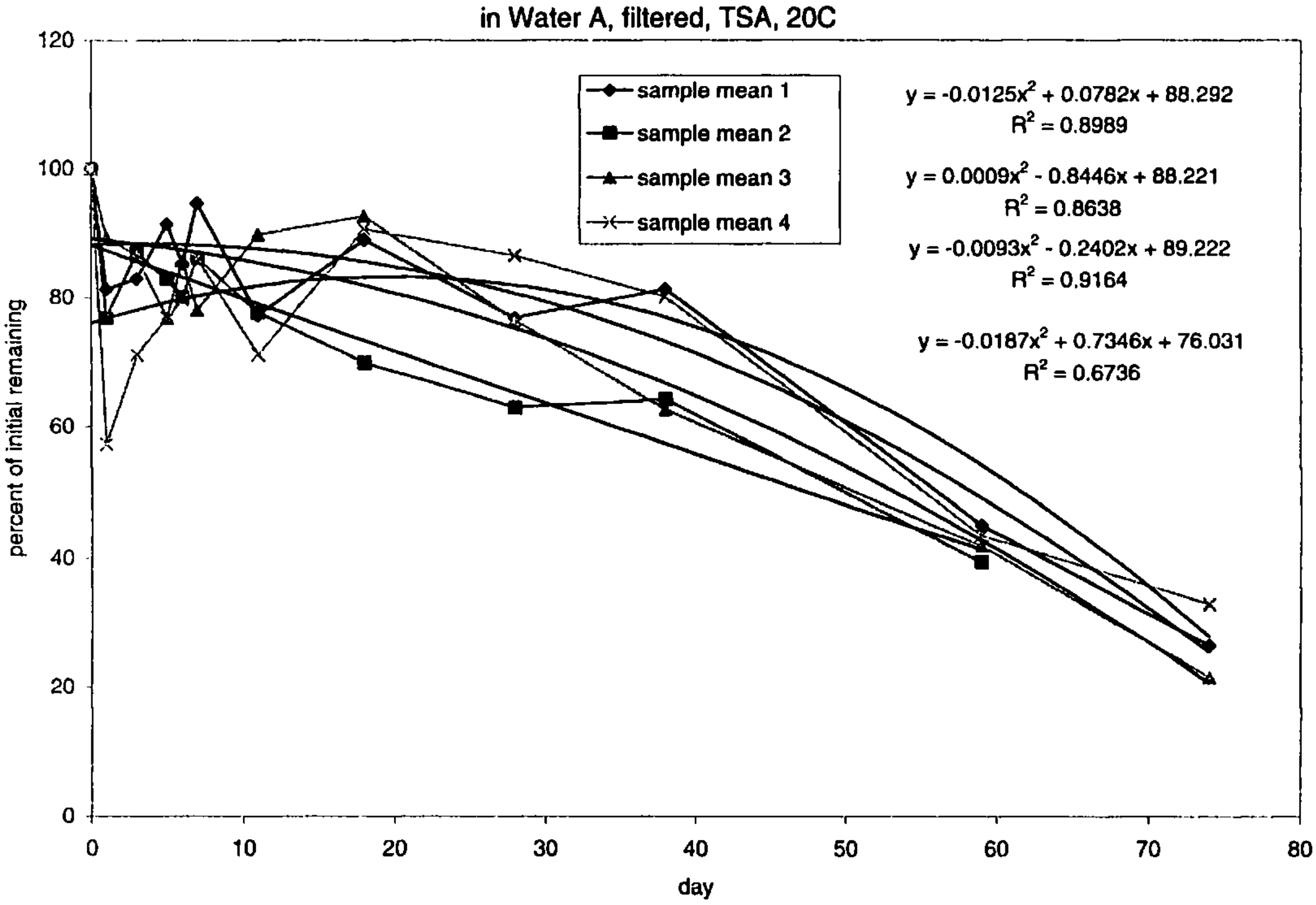
Appendix 54. Decline phase of E. coli O157:H7 bovine isolate, in Water A, filtered, TSA, 10C



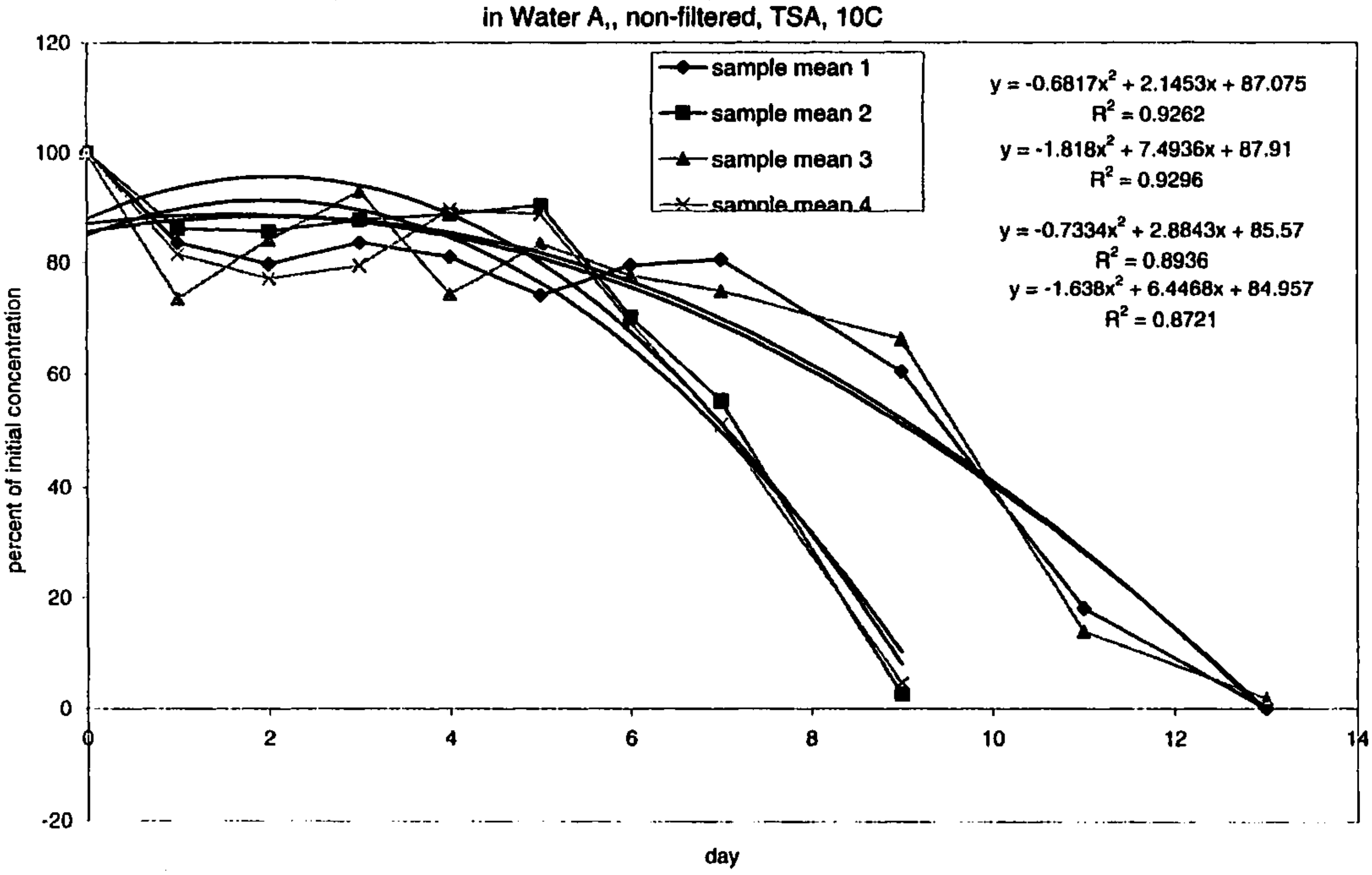
Appendix 55. Decline phase of S. typhimurium laboratory isolate, i in Water A, filtered, TSA, 20C

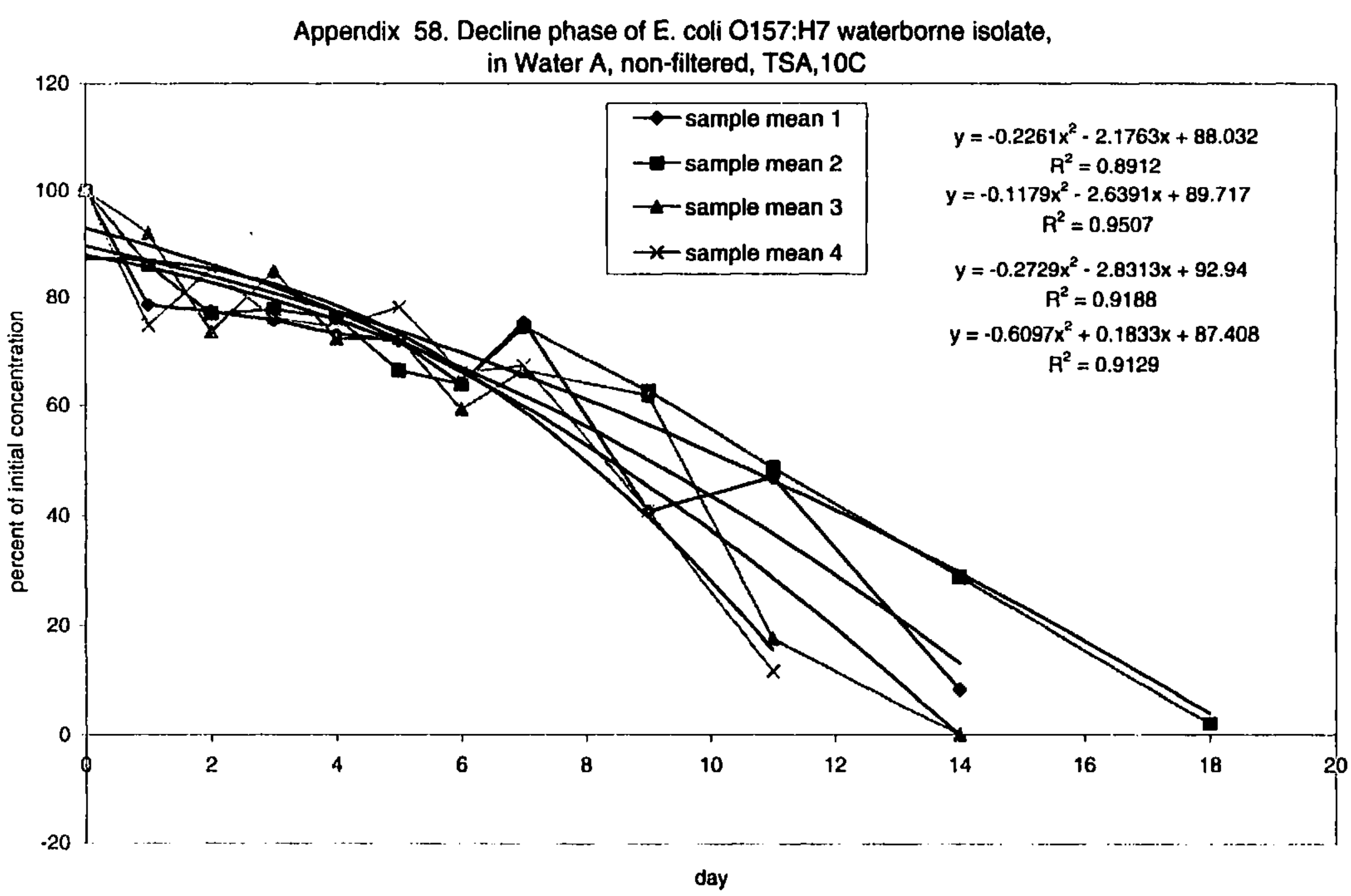


Appendix 56. Decline phase of E. coli O157:H7 bovine isolate, in Water A, filtered, TSA, 20C

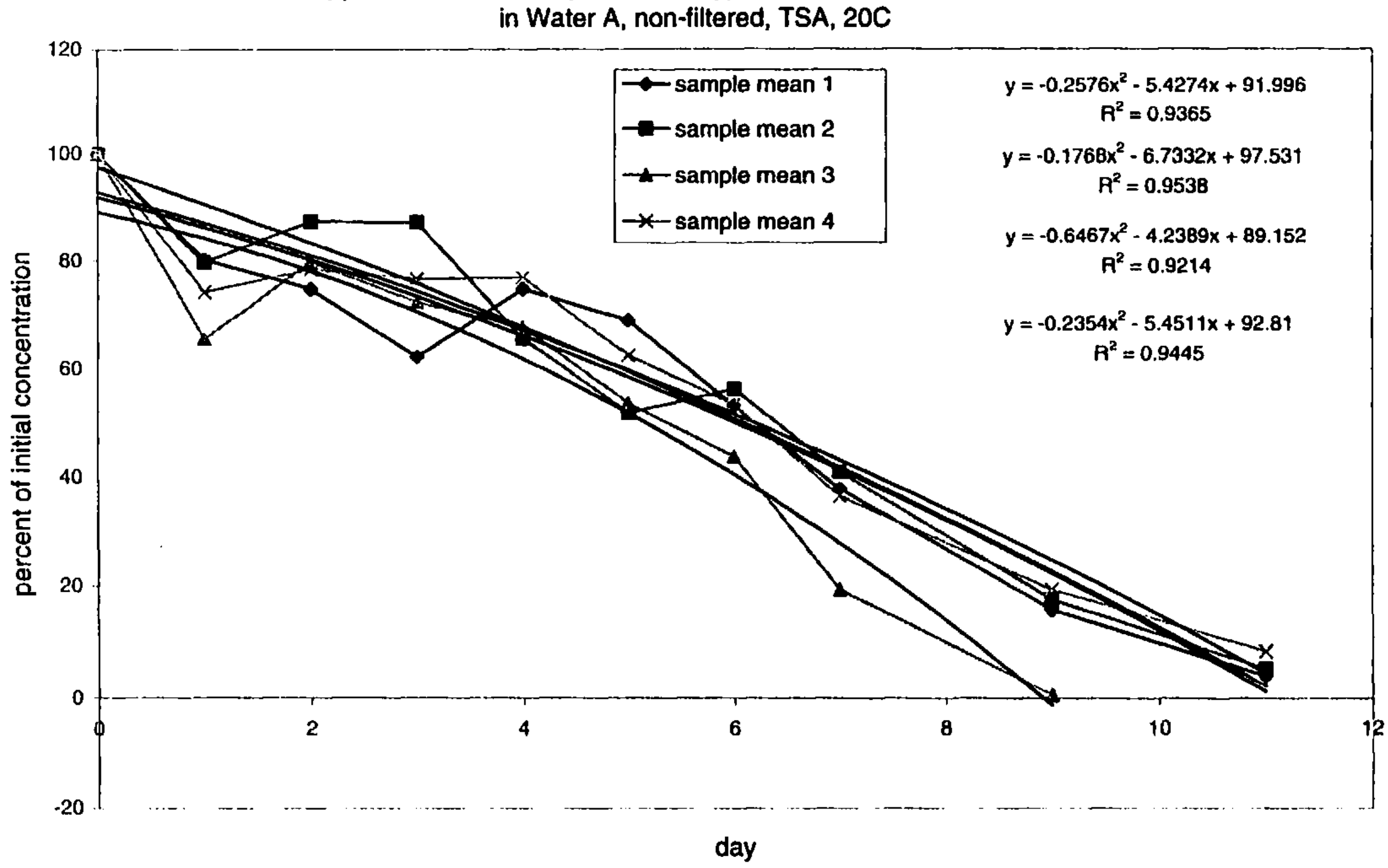


Appendix 57. Decline phase of S. typhimurium waterborne isolate, in Water A,, non-filtered, TSA, 10C

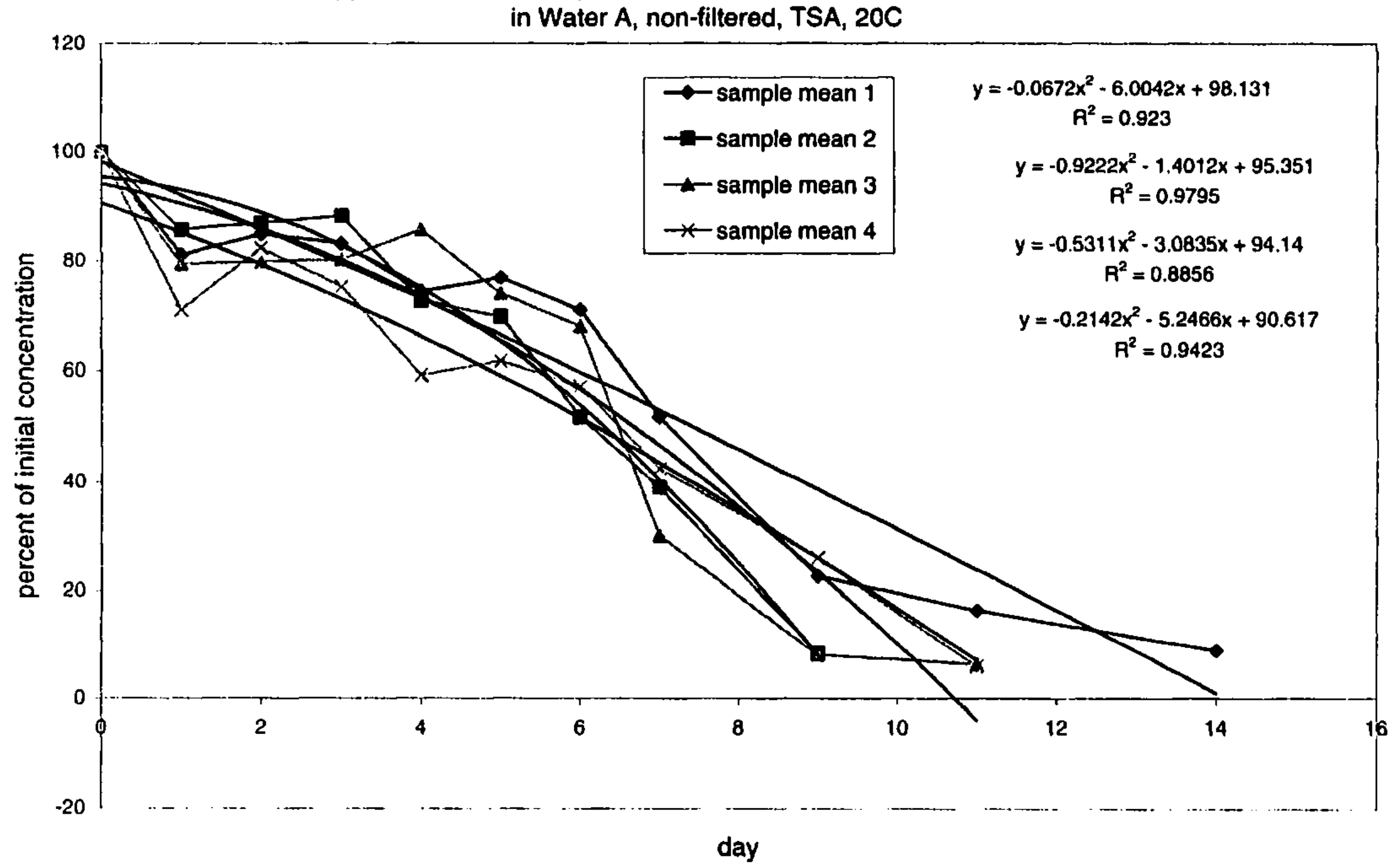



Appendix 58. Decline phase of E. coli O157:H7 waterborne isolate,
in Water A, non-filtered, TSA,10C
sample mean 1
sample mean 2
sample mean 3
sample mean 4
y = -0.2261x² - 2.1763x + 88.032
R² = 0.8912
y = -0.1179x² - 2.6391x + 89.717
R² = 0.9507
y = -0.2729x² - 2.8313x + 92.94
R² = 0.9188
y = -0.6097x² + 0.1833x + 87.408
R² = 0.9129
percent of initial concentration
day

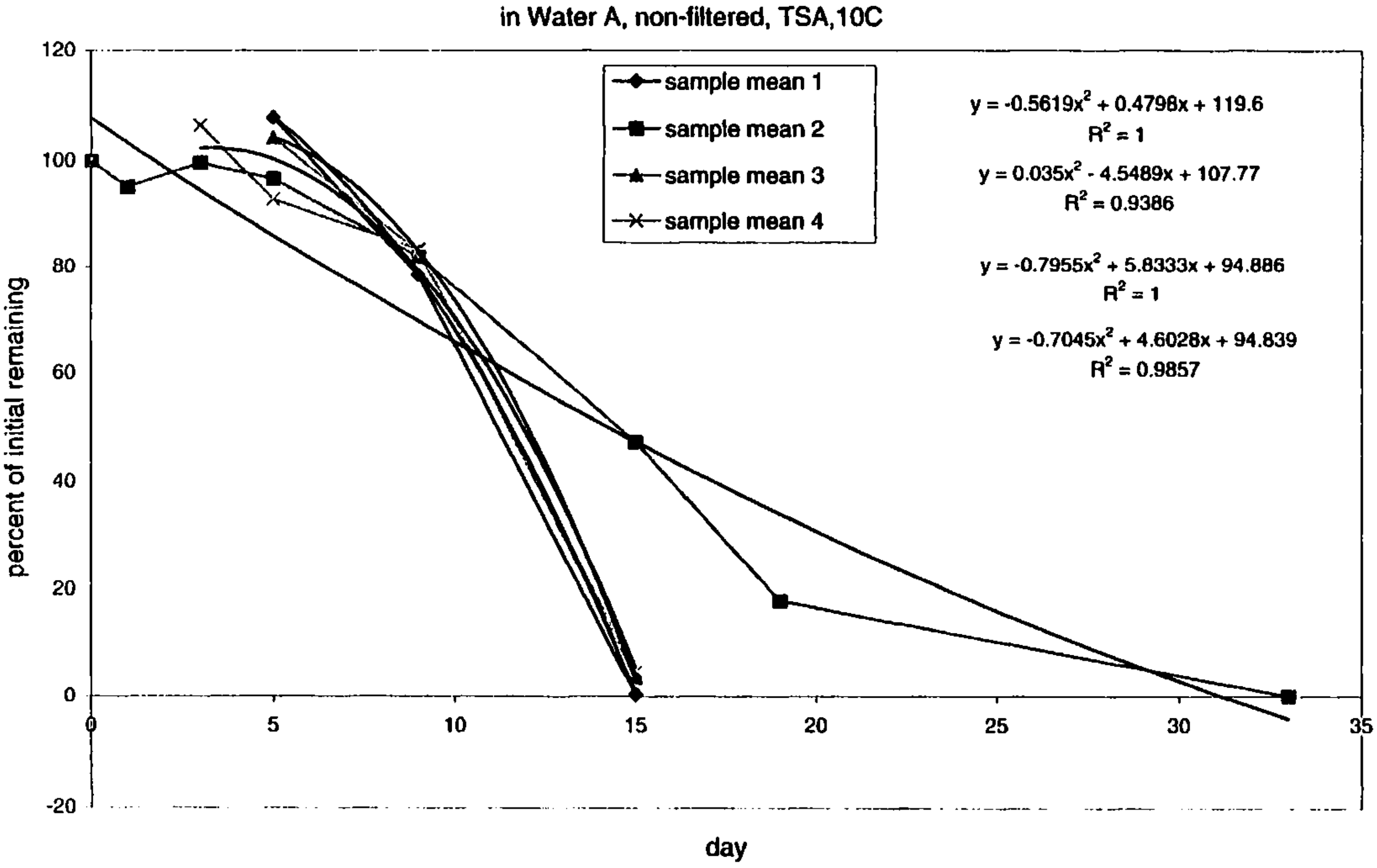
Appendix 59. Decline phase of S. typhimurium waterborne isolate, in Water A, non-filtered, TSA, 20C



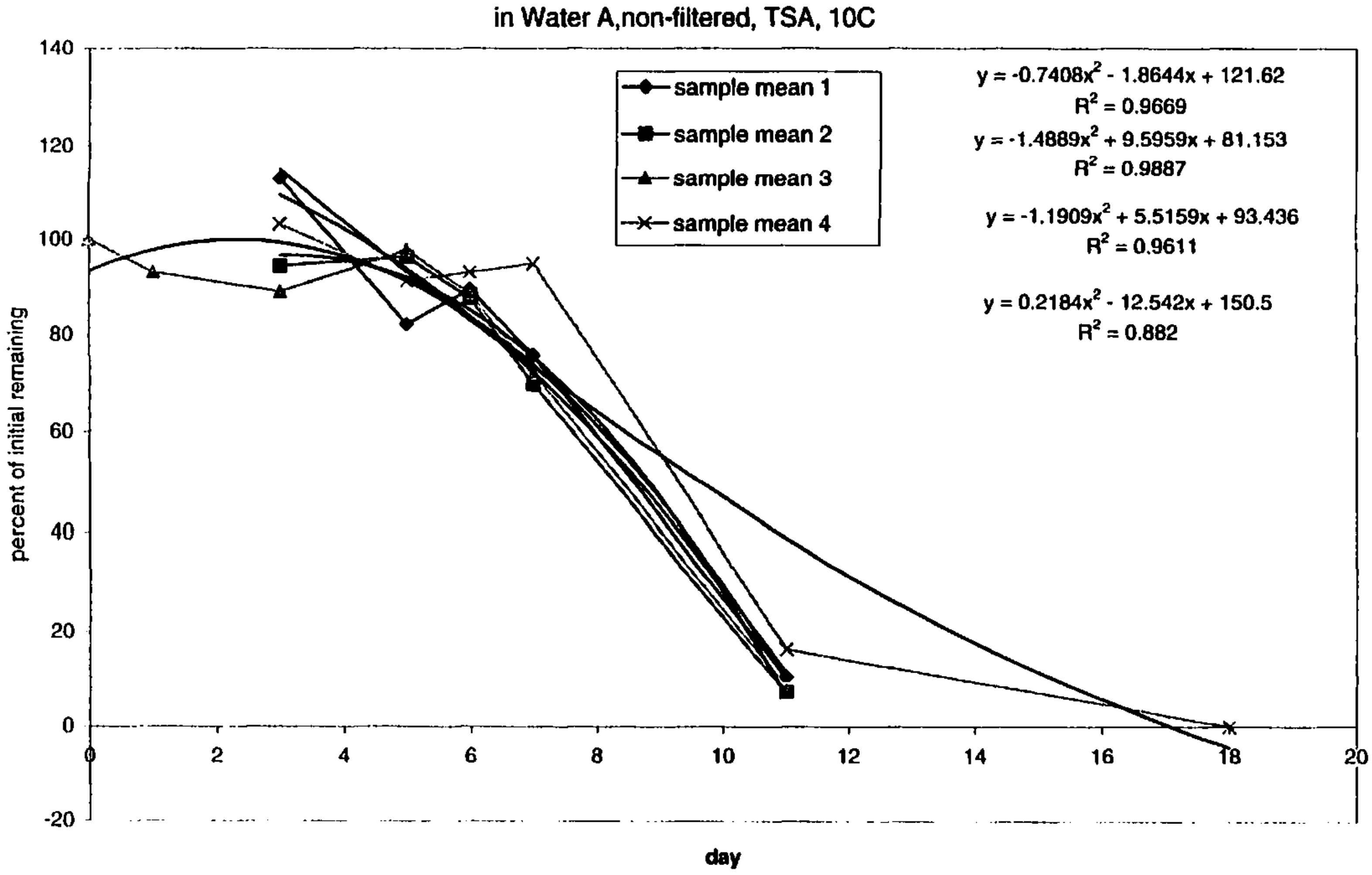
Appendix 60. Decline phase of E. coli O157:H7 waterborne isolate, in Water A, non-filtered, TSA, 20C



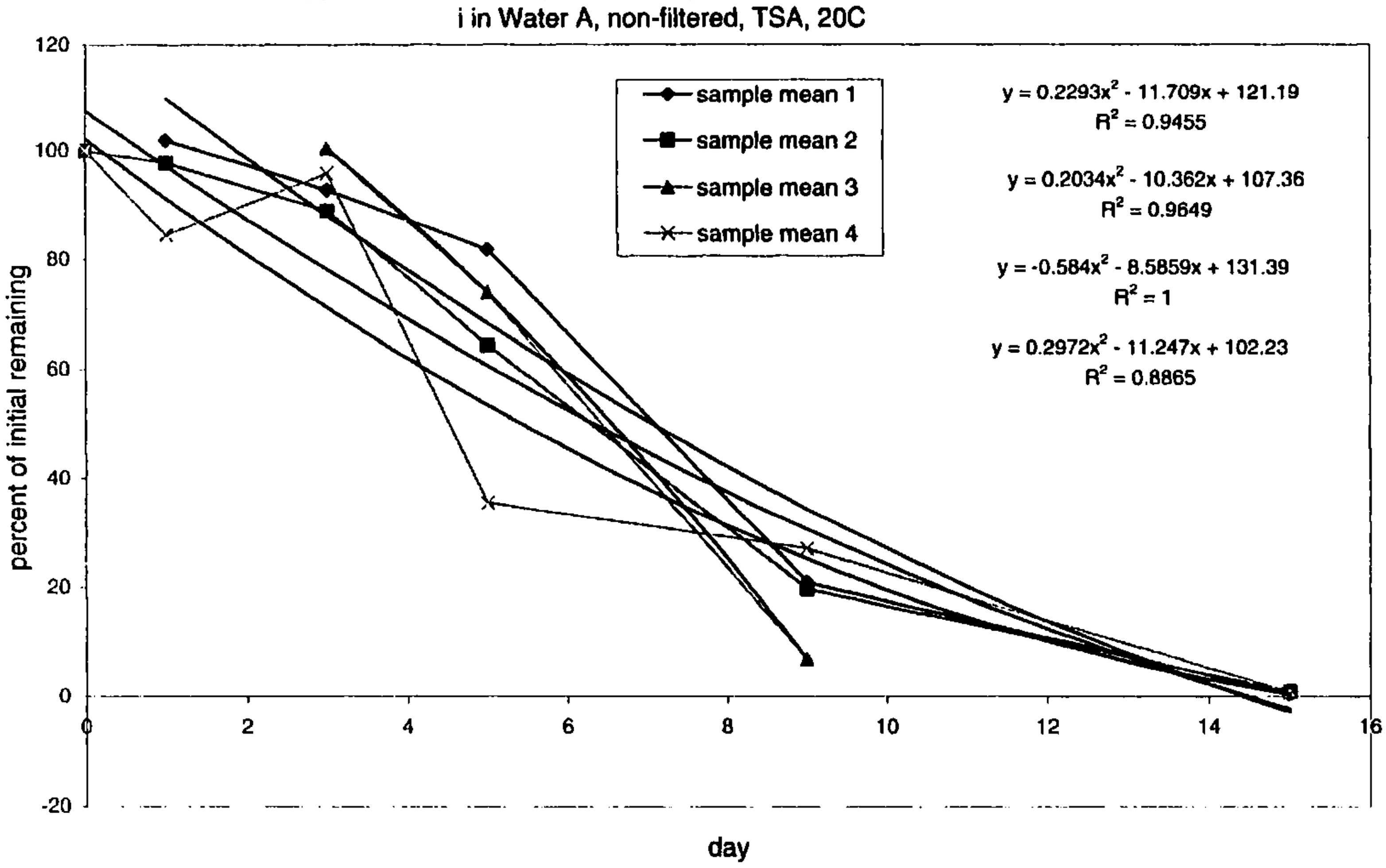
Appendix 61. Decline phase of S. typhimurium laboratory isolate, in Water A, non-filtered, TSA,10C



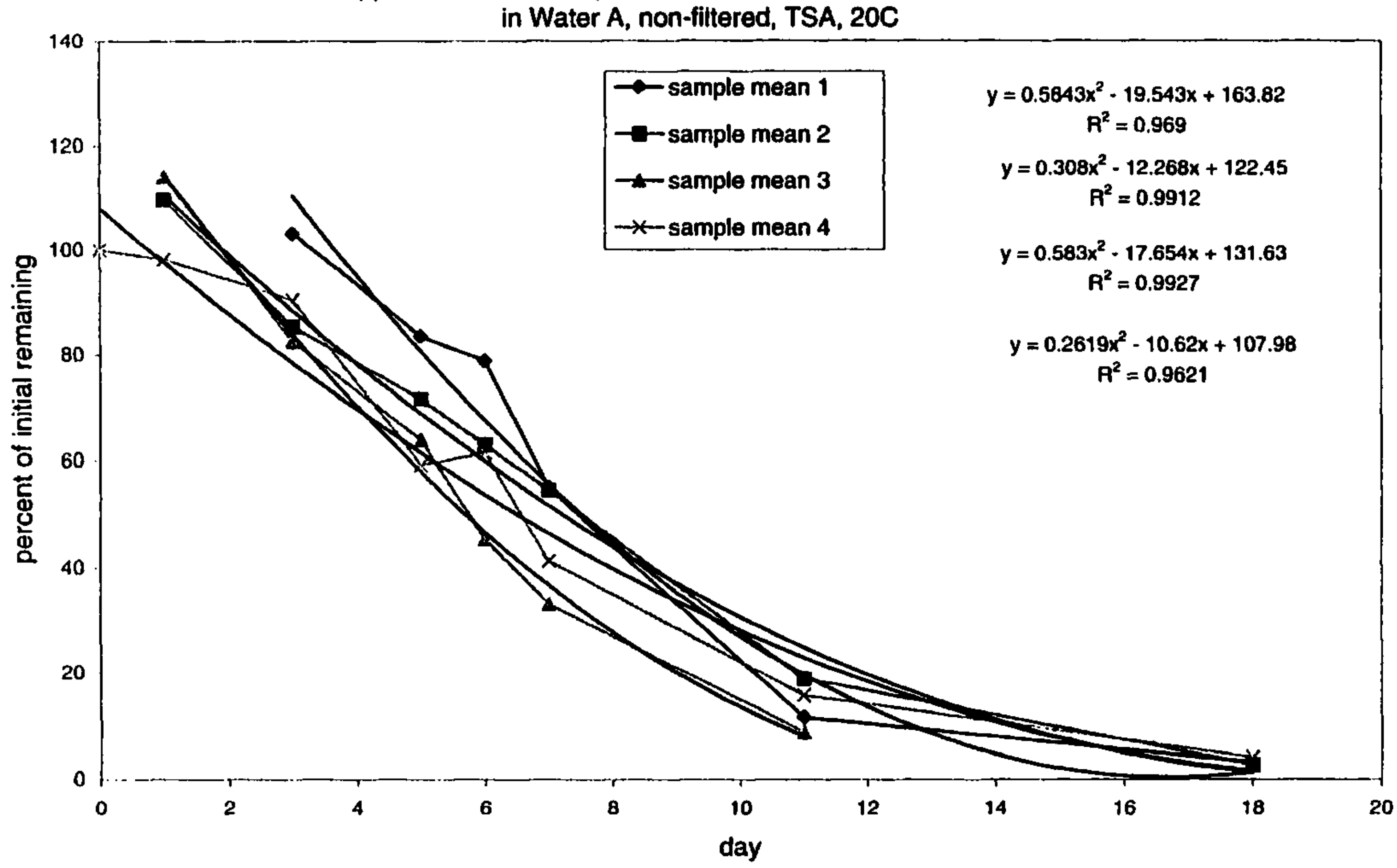
Appendix 62. Decline phase of E. coliO157:H7 bovine isolate, in Water A,non-filtered, TSA, 10C



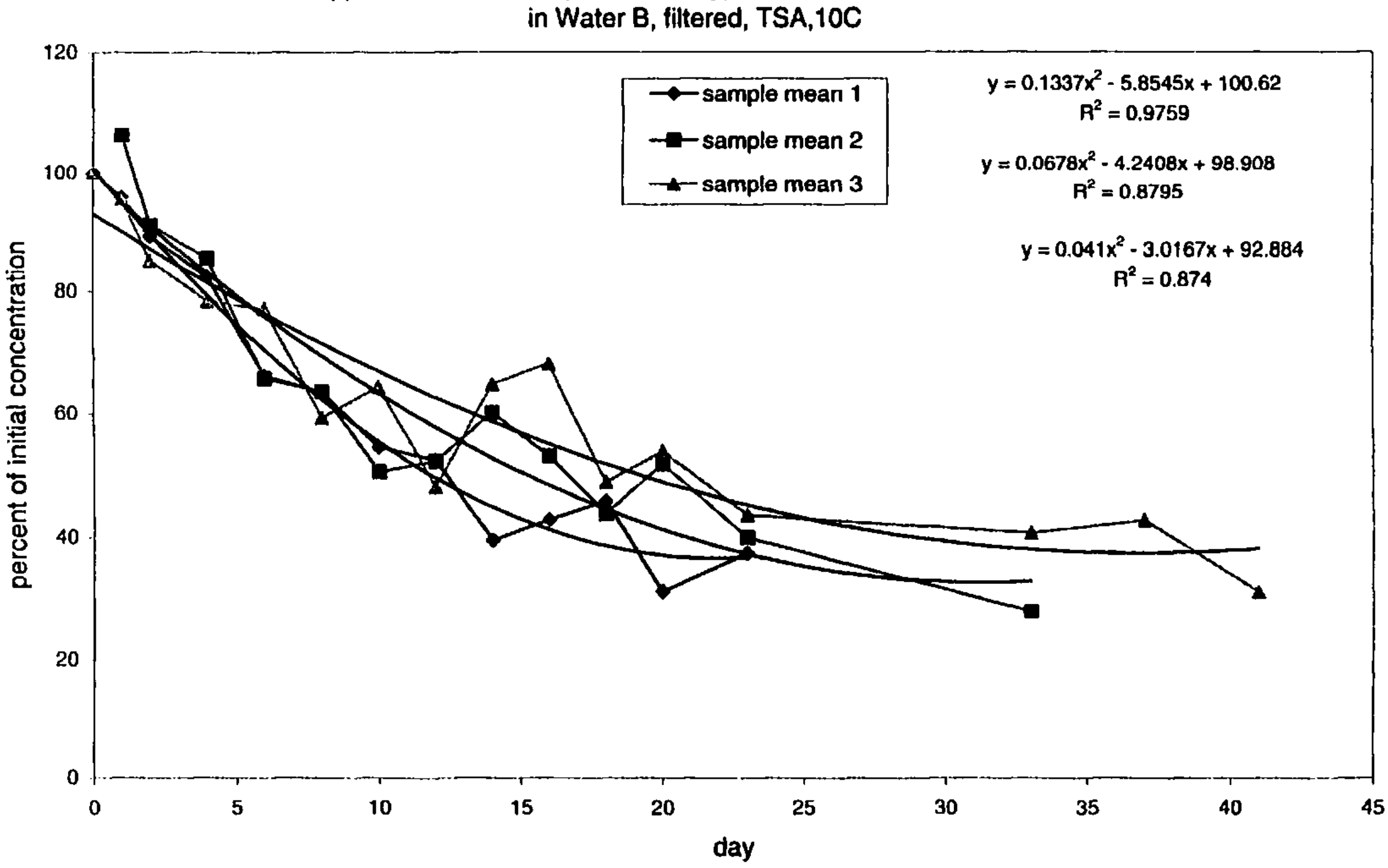
Appendix 63. Decline phase of S. typhimurium laboratory isolate, i in Water A, non-filtered, TSA, 20C



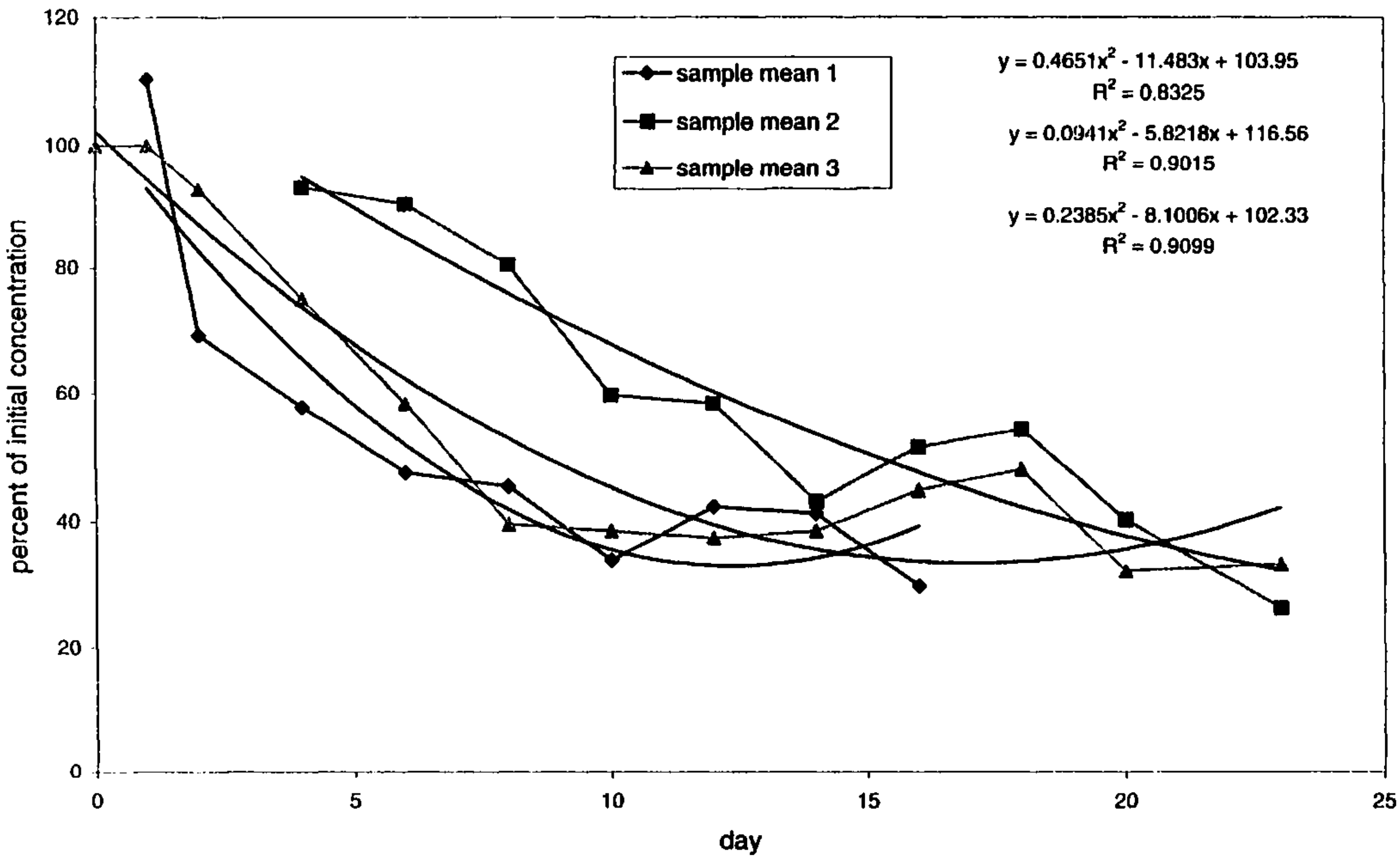
Appendix 64. Decline phase of E. coliO157:H7 bovine isolate,
in Water A, non-filtered, TSA, 20C



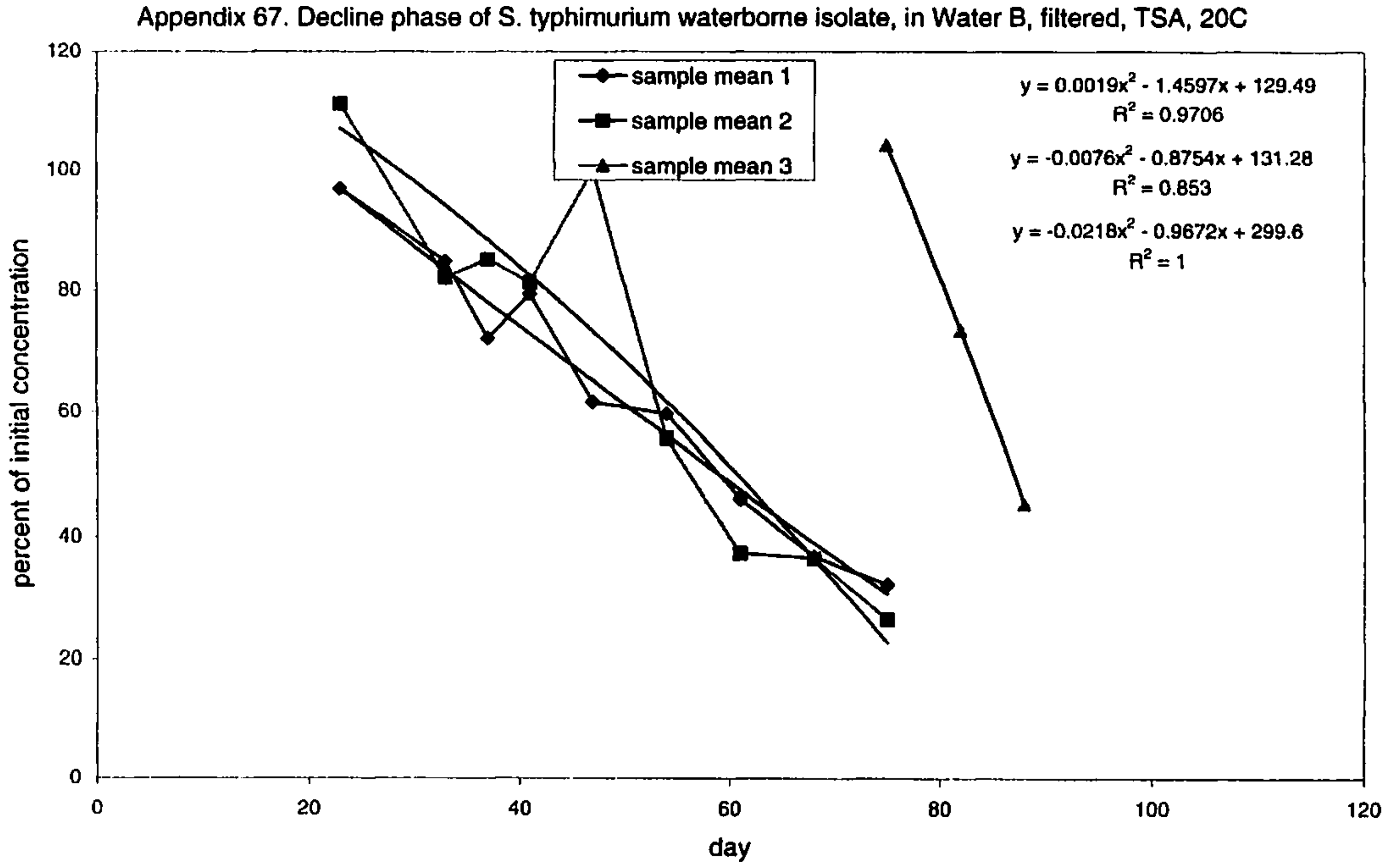
Appendix 65. Decline phase of S. typhimurium waterborne isolate, in Water B, filtered, TSA,10C



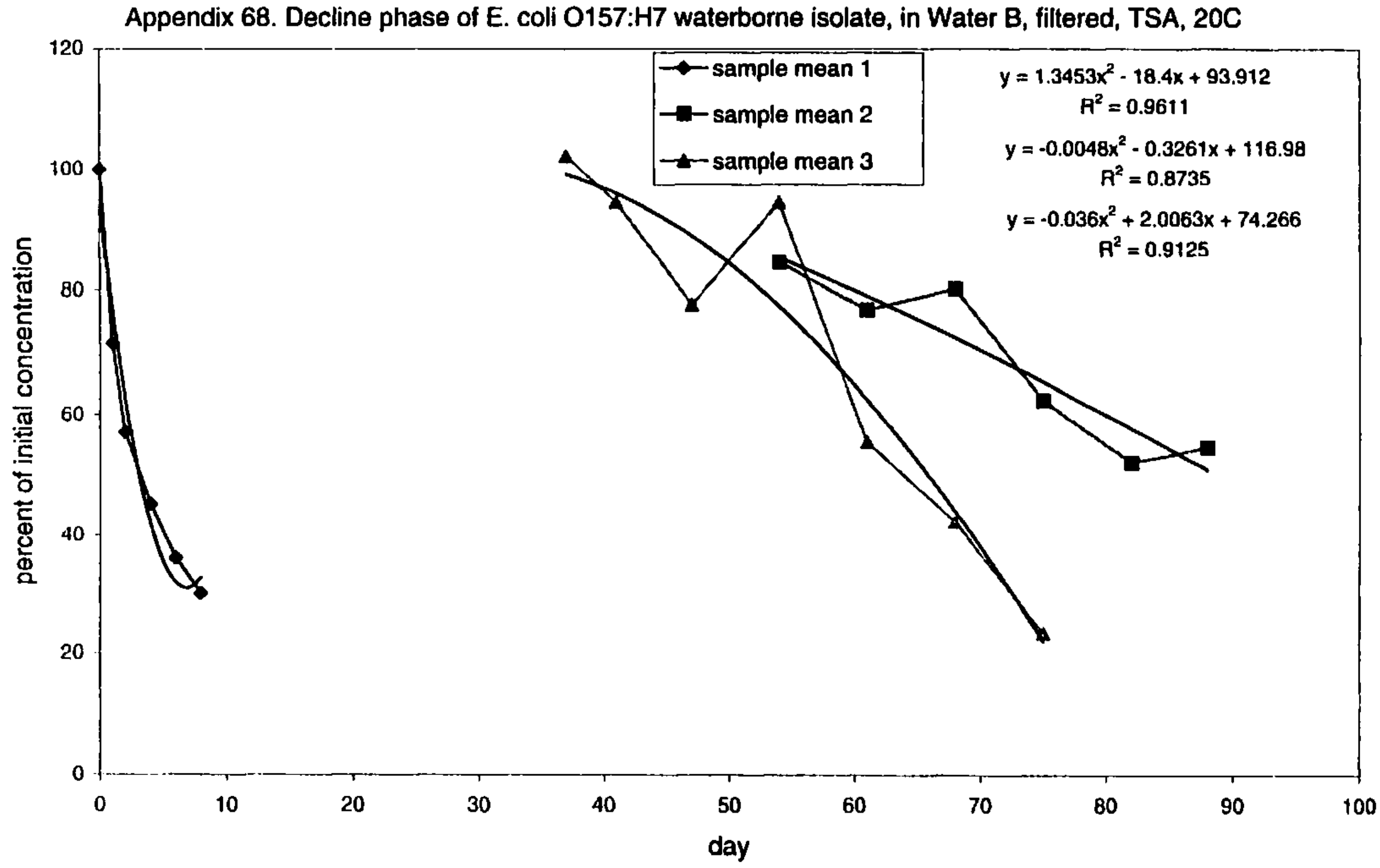
Appendix 66. Decline phase of E. coli O157:H7 waterborne isolate, in Water B, filtered, TSA, 10C

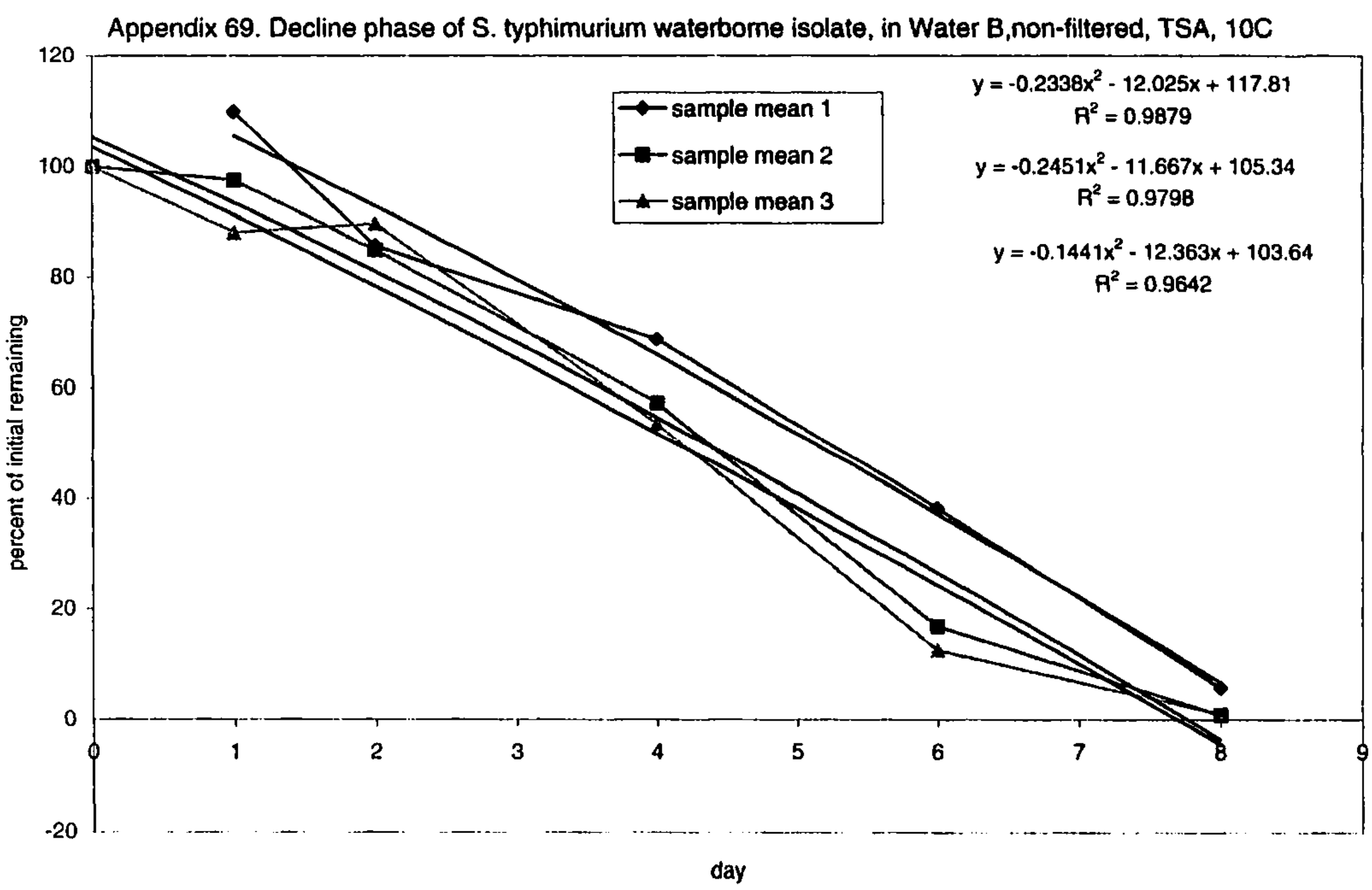


Appendix 67. Decline phase of S. typhimurium waterborne isolate, in Water B, filtered, TSA, 20C



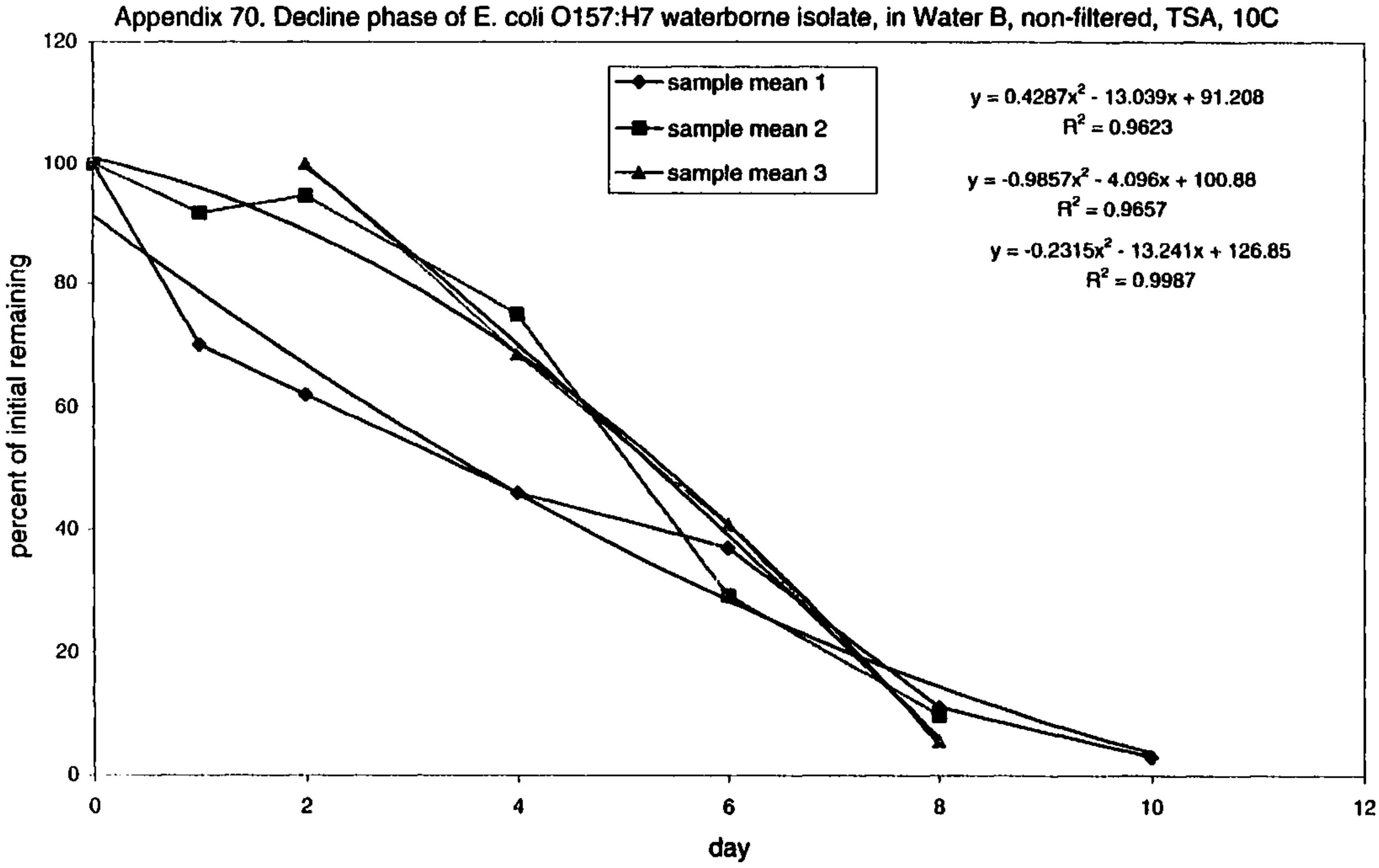
Appendix 68. Decline phase of E. coli O157:H7 waterborne isolate, in Water B, filtered, TSA, 20C



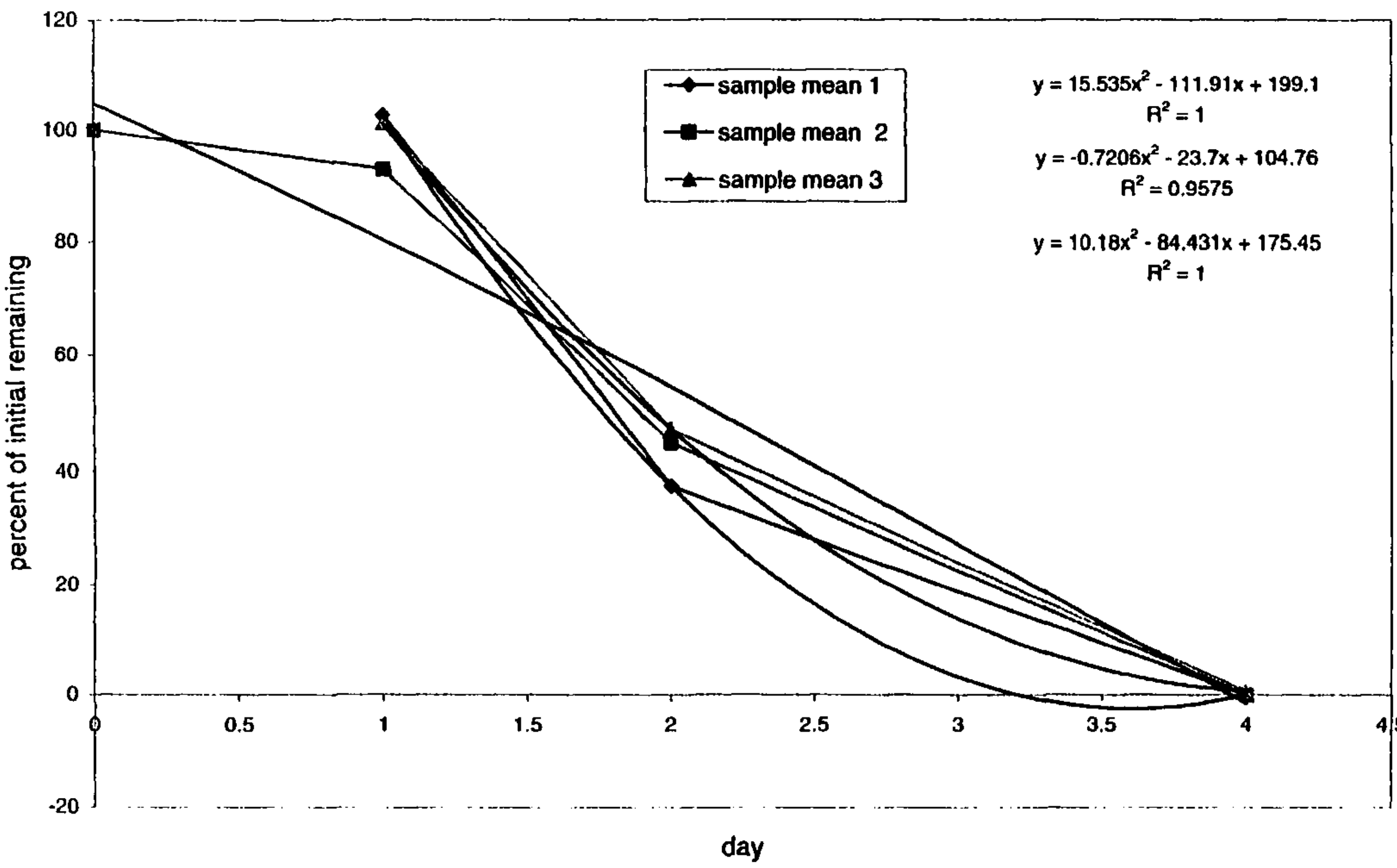


Appendix 69. Decline phase of S. typhimurium waterborne isolate, in Water B,non-filtered, TSA, 10C
sample mean 1
sample mean 2
sample mean 3
y = -0.2338x2 - 12.025x + 117.81
R2 = 0.9879
y = -0.2451x2 - 11.667x + 105.34
R2 = 0.9798
y = -0.1441x2 - 12.363x + 103.64
R2 = 0.9642
percent of initial remaining
day
120
100
80
60
40
20
0
-20
0
1
2
3
4
5
6
7
8
9

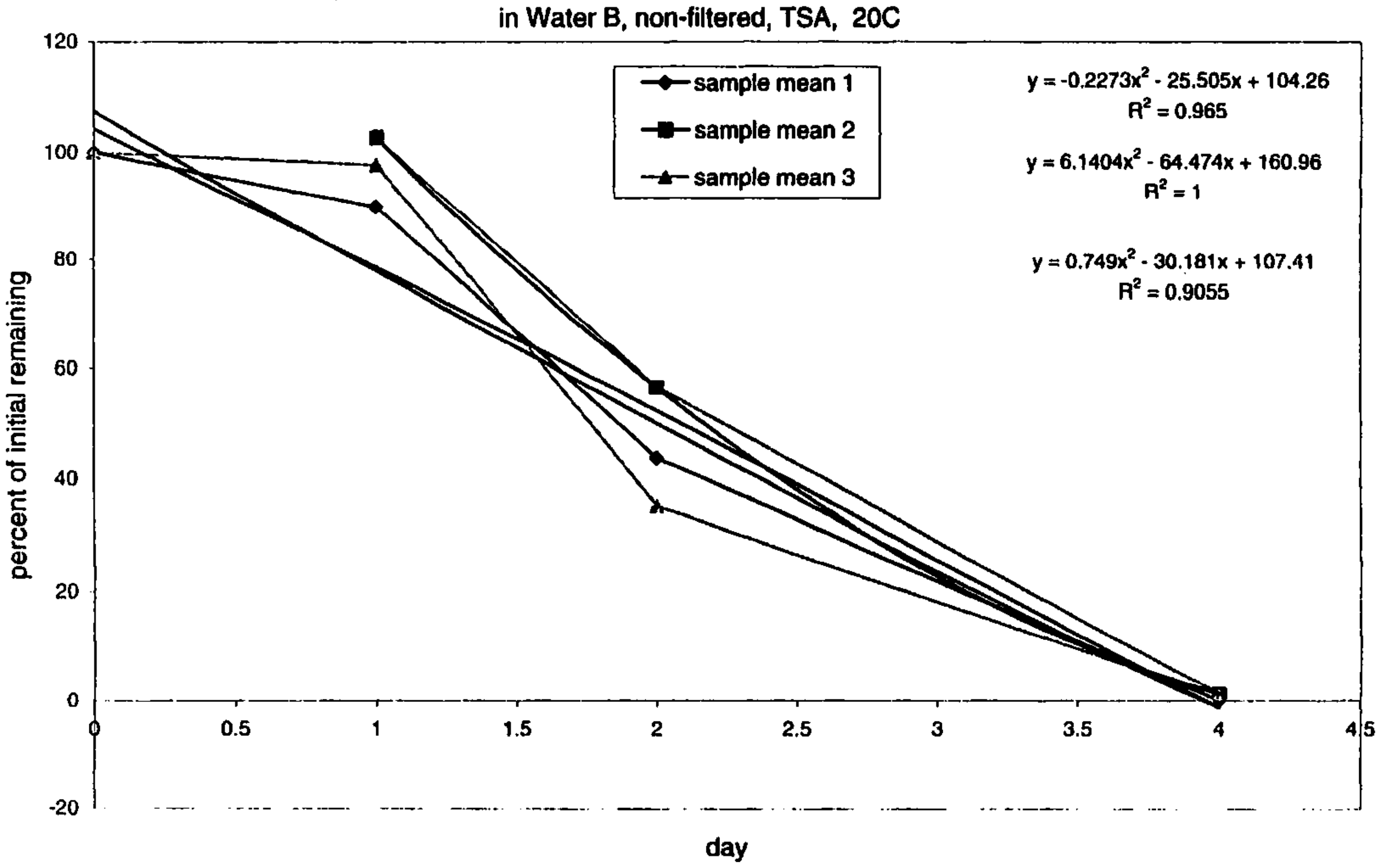
Appendix 70. Decline phase of E. coli O157:H7 waterborne isolate, in Water B, non-filtered, TSA, 10C



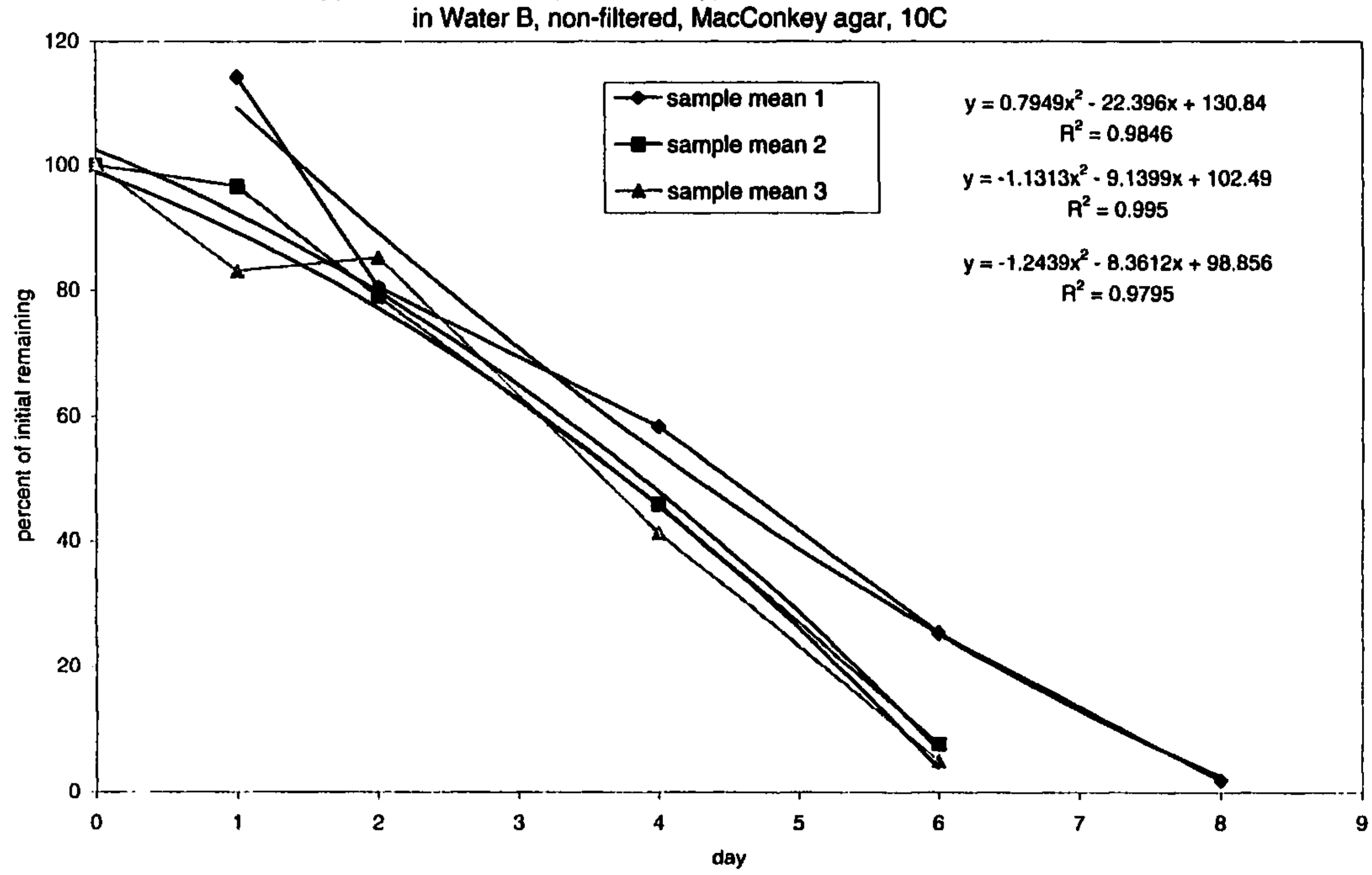
Appendix 71. Decline phase of S. typhimurium waterborne isolate, in Water B, non-filtered, TSA, 20C



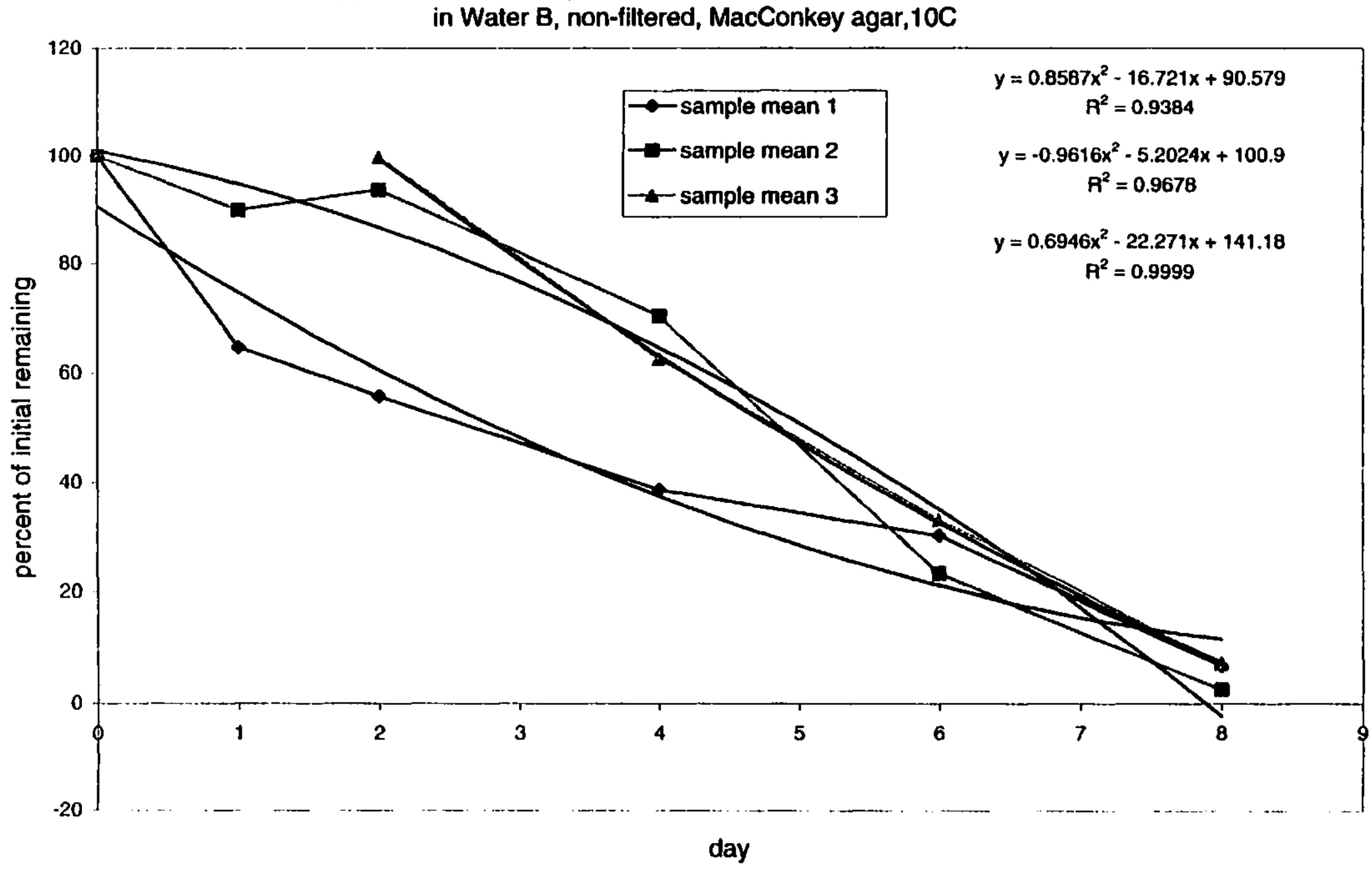
Appendix 72. Decline phase of E. coli O157:H7 waterborne isolate, in Water B, non-filtered, TSA, 20C



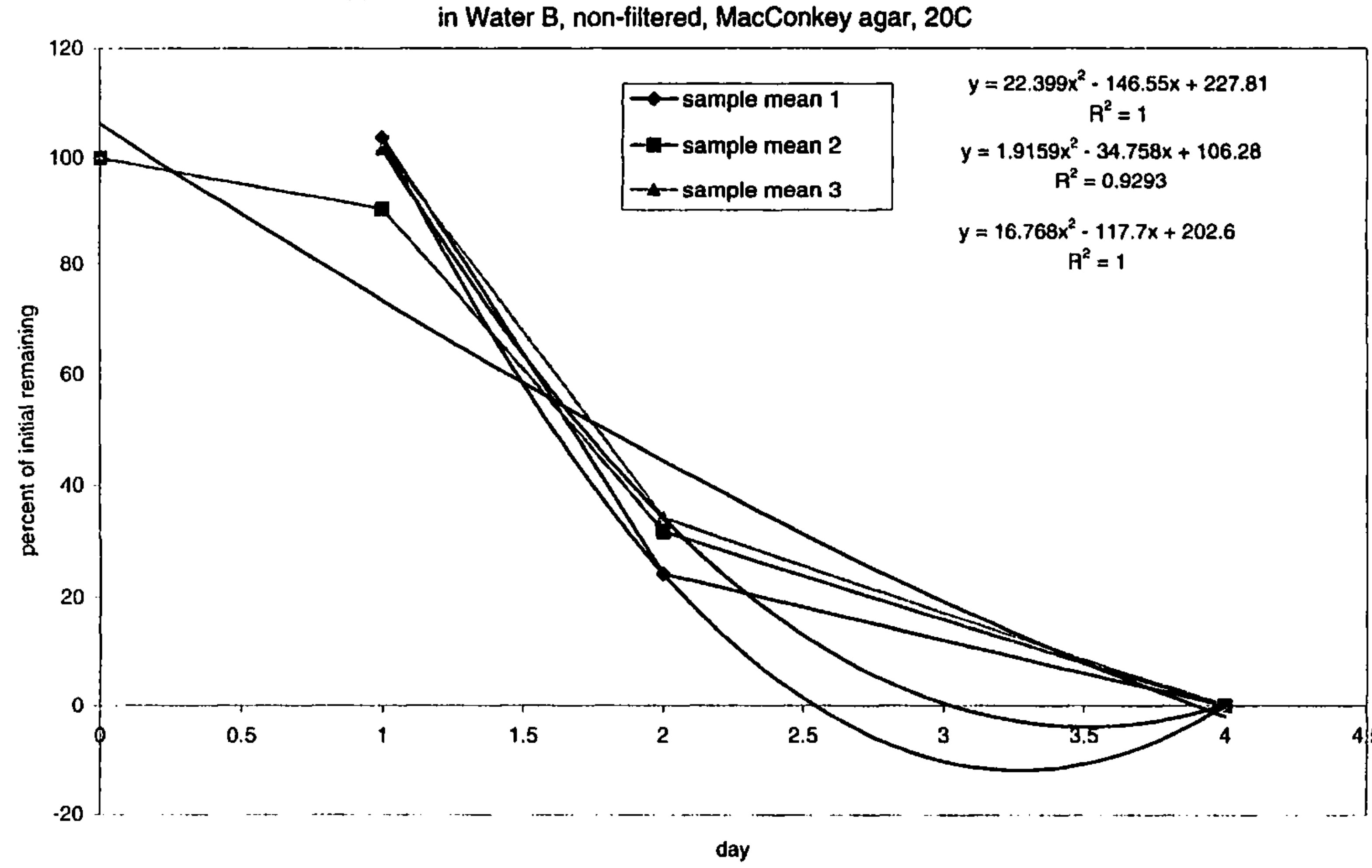
Appendix 73. Decline phase of S. typhimurium waterborne isolate, in Water B, non-filtered, MacConkey agar, 10C



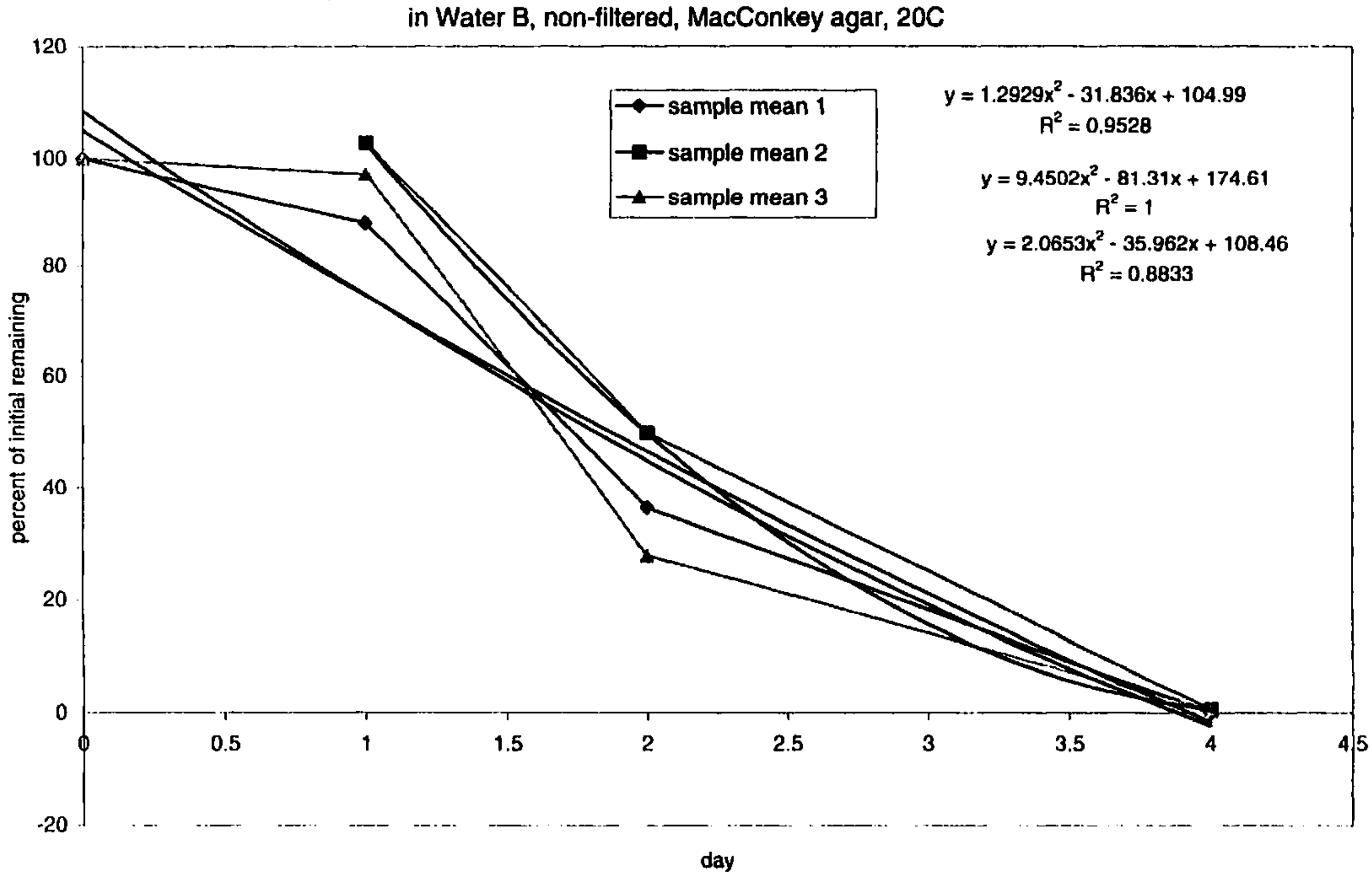
Appendix 74. Decline phase of E. coli O157:H7 waterborne isolate, in Water B, non-filtered, MacConkey agar,10C



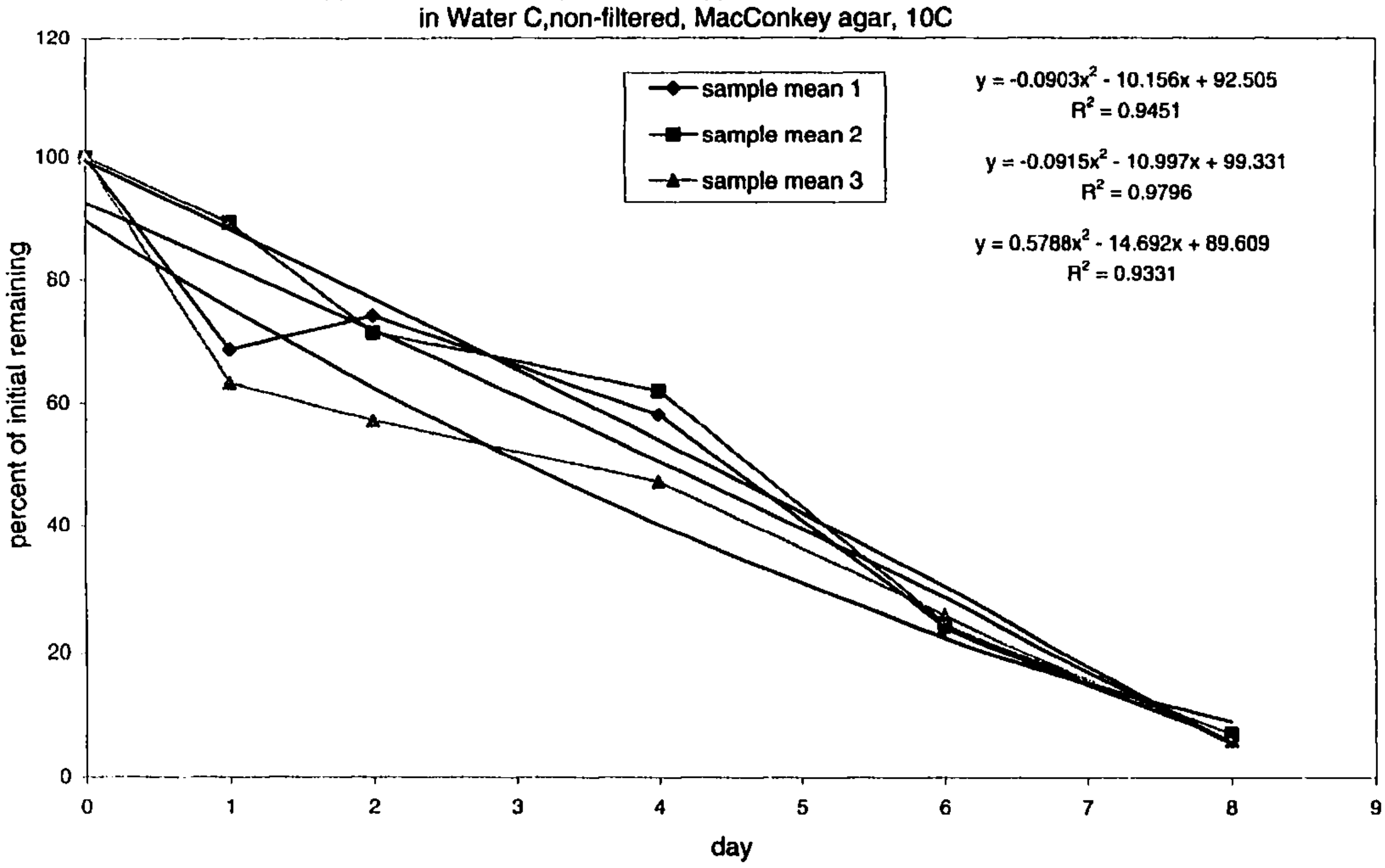
Appendix 75. Decline phase of S. typhimurium waterborne isolate, in Water B, non-filtered, MacConkey agar, 20C



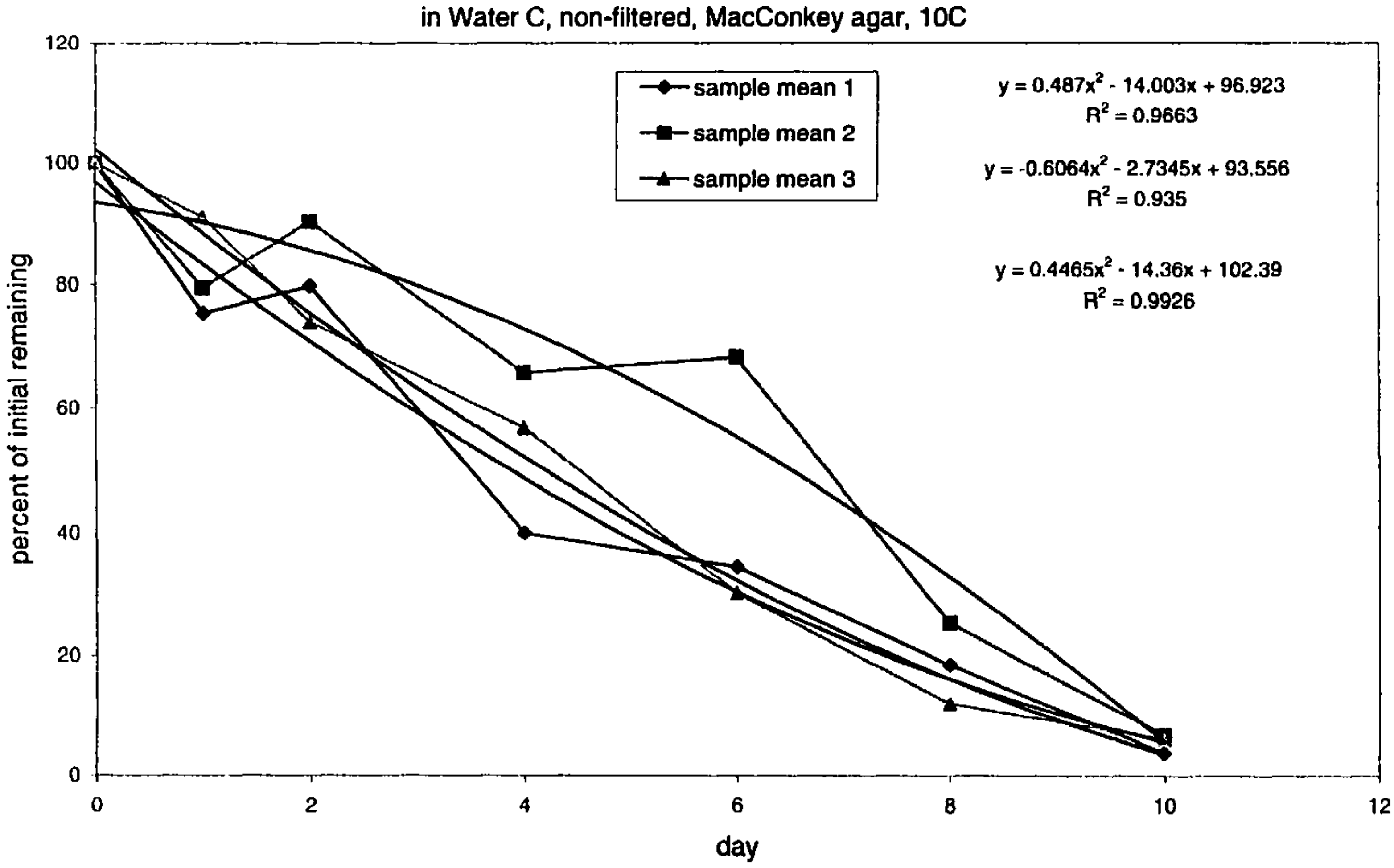
Appendix 76. Decline phase of E. coli O157:H7 waterborne isolate,
in Water B, non-filtered, MacConkey agar, 20C



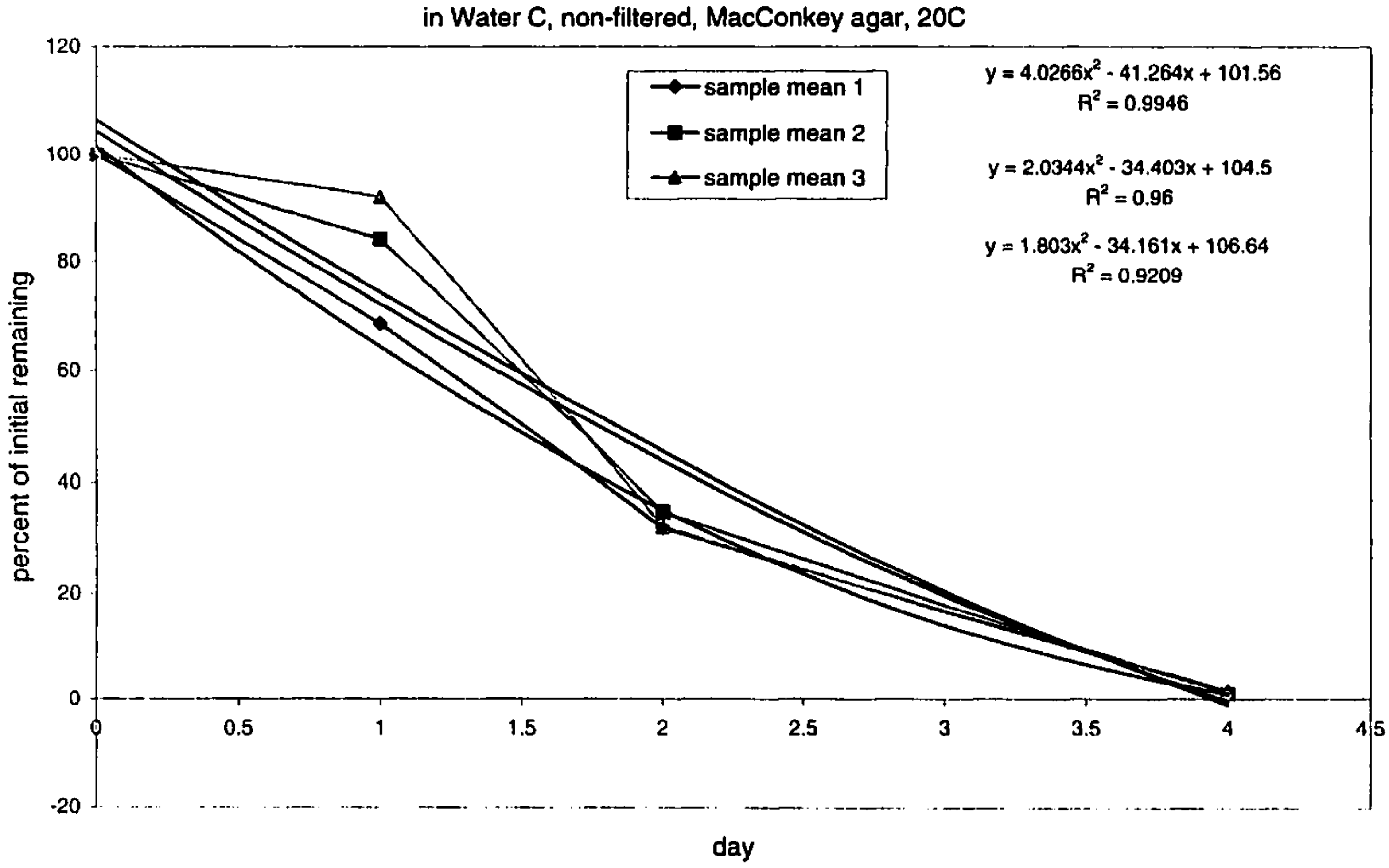
Appendix 77. Decline phase of S. typhimurium waterborne isolate, in Water C,non-filtered, MacConkey agar, 10C



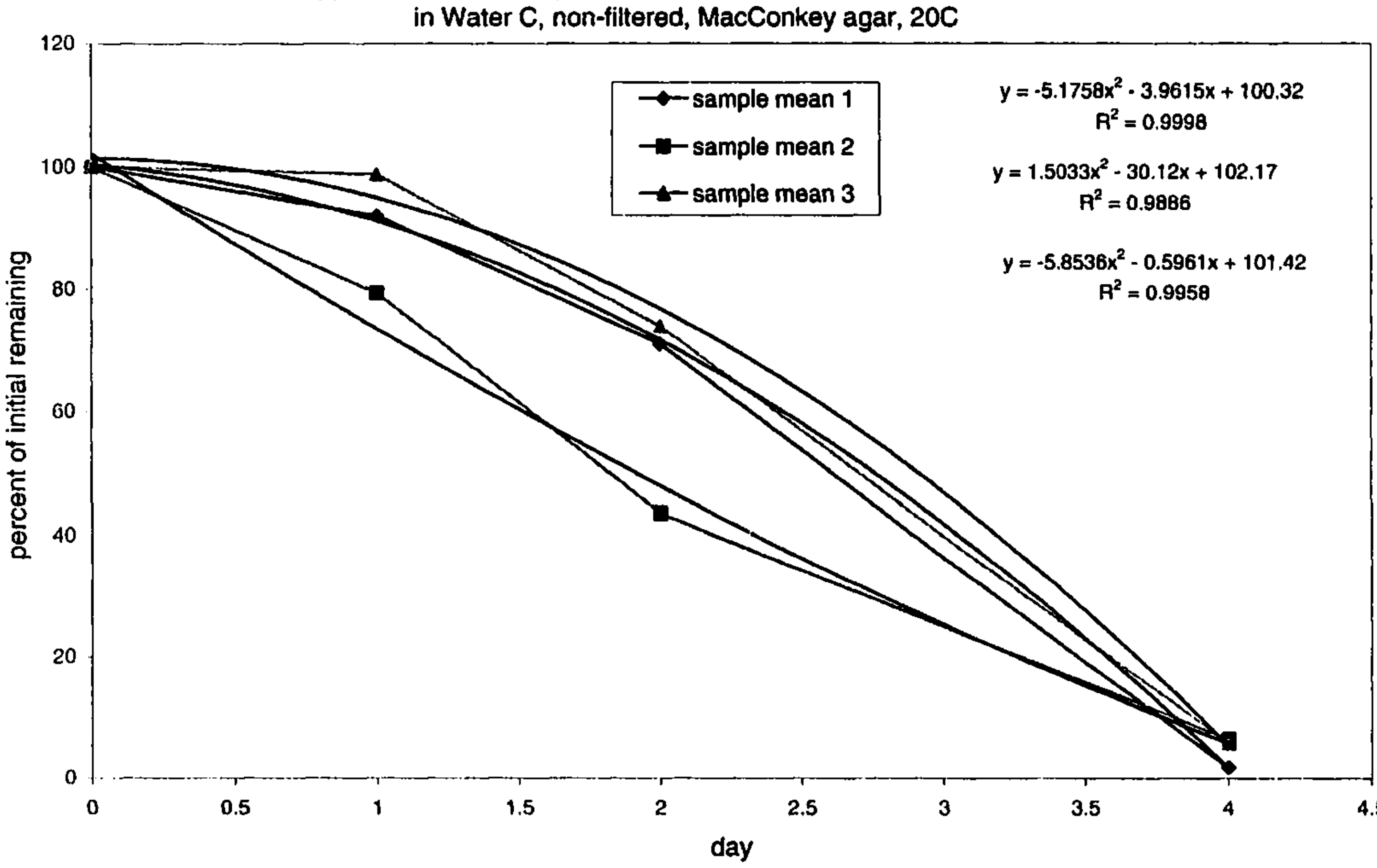
Appendix 78. Decline phase of E. coli O157:H7 waterborne isolate, in Water C, non-filtered, MacConkey agar, 10C



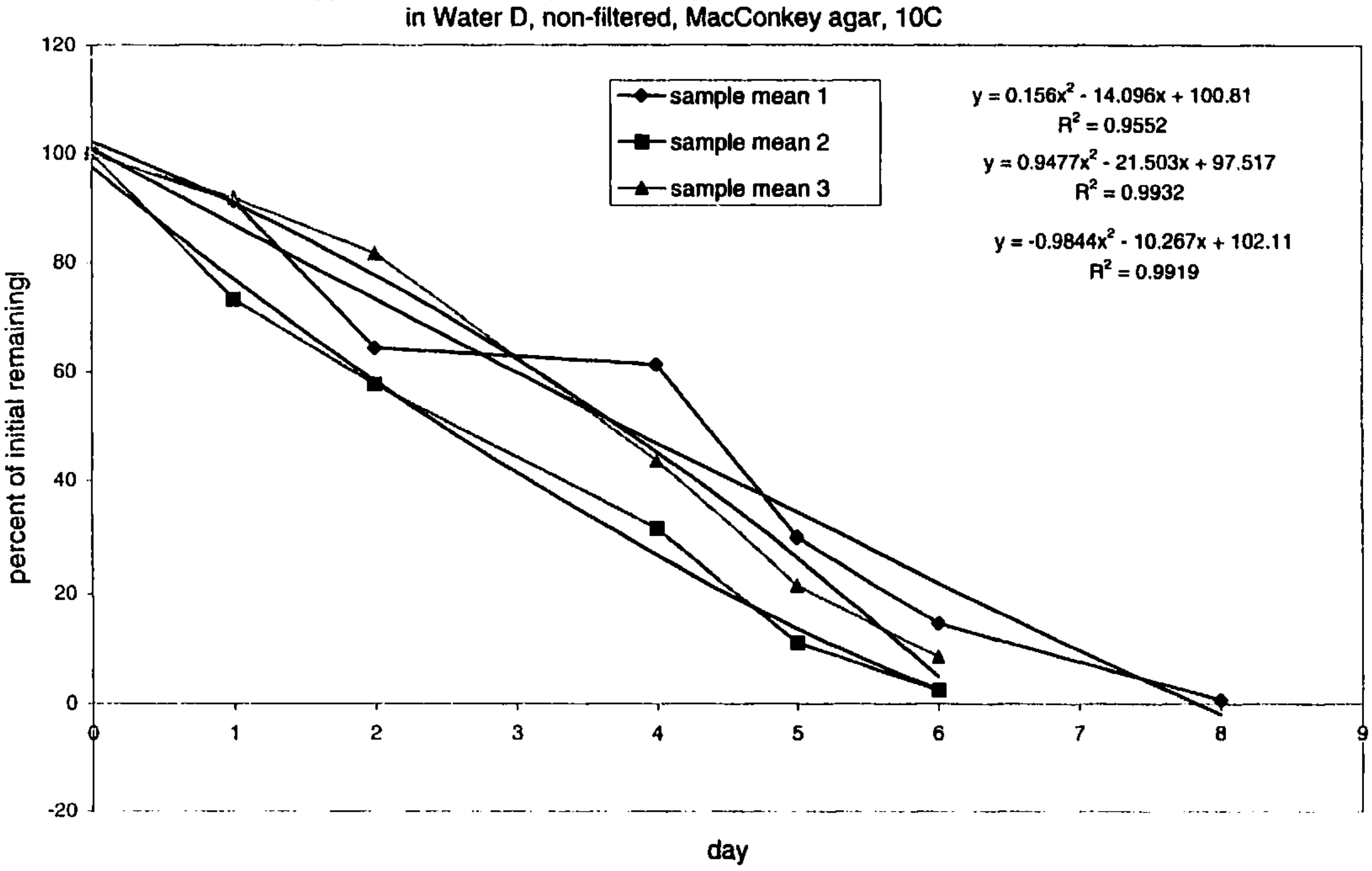
Appendix 79. Decline phase of S. typhimurium waterborne isolate, in Water C, non-filtered, MacConkey agar, 20C



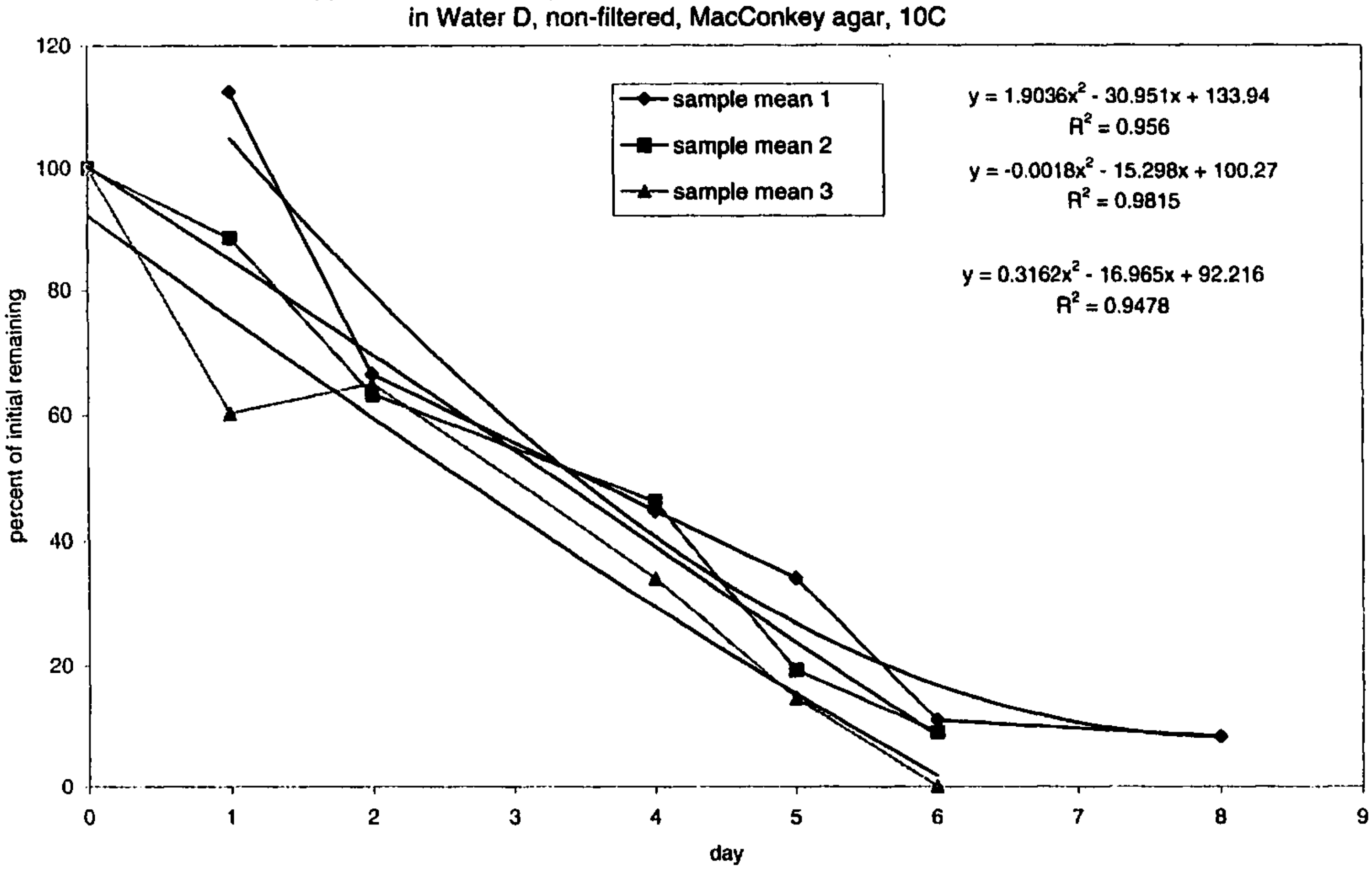
Appendix 80. Decline phase of E. coli O157:H7 waterborne isolate, in Water C, non-filtered, MacConkey agar, 20C



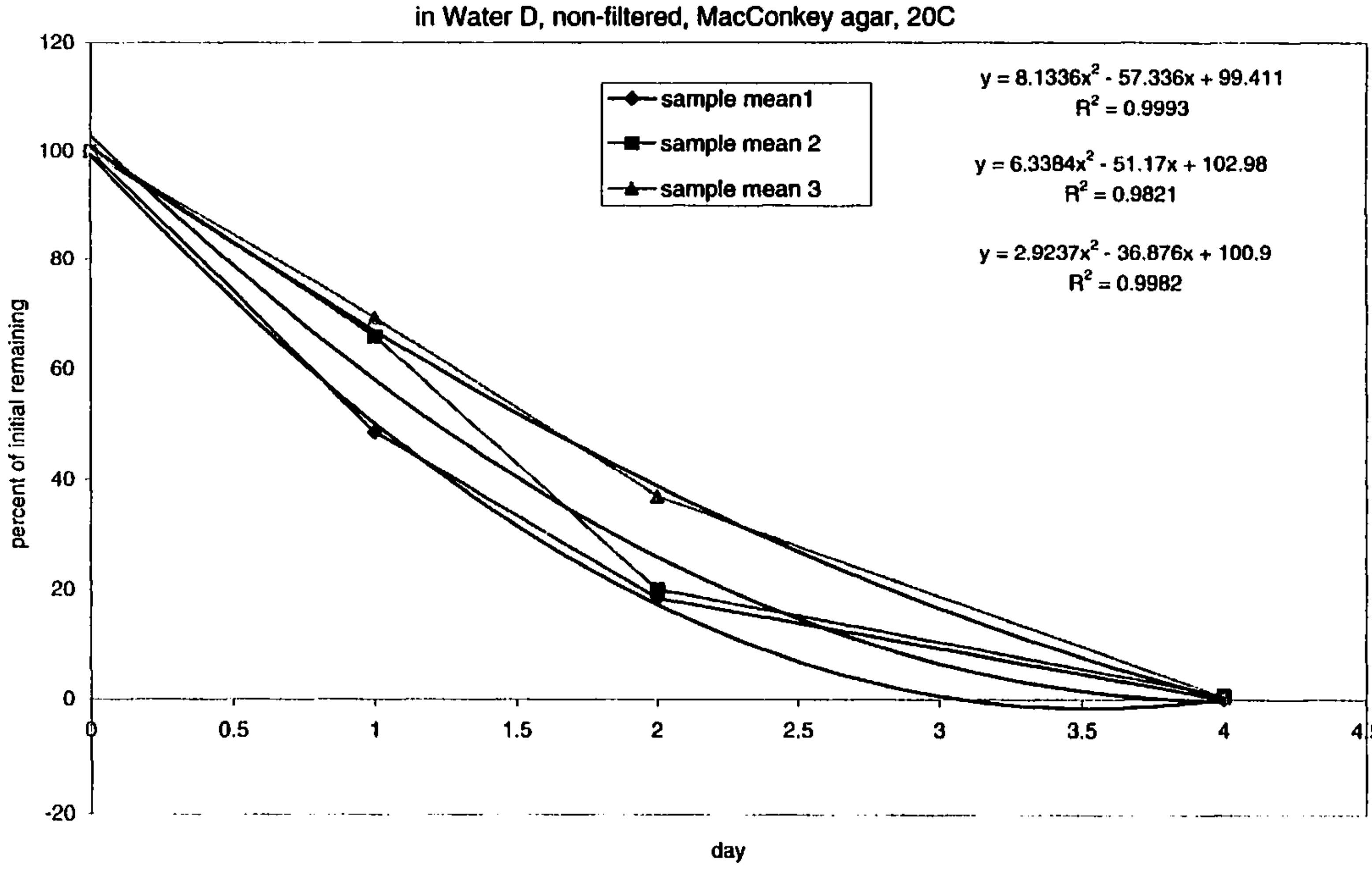
Appendix 81. Decline phase of S. typhimurium waterborne isolate,
in Water D, non-filtered, MacConkey agar, 10C



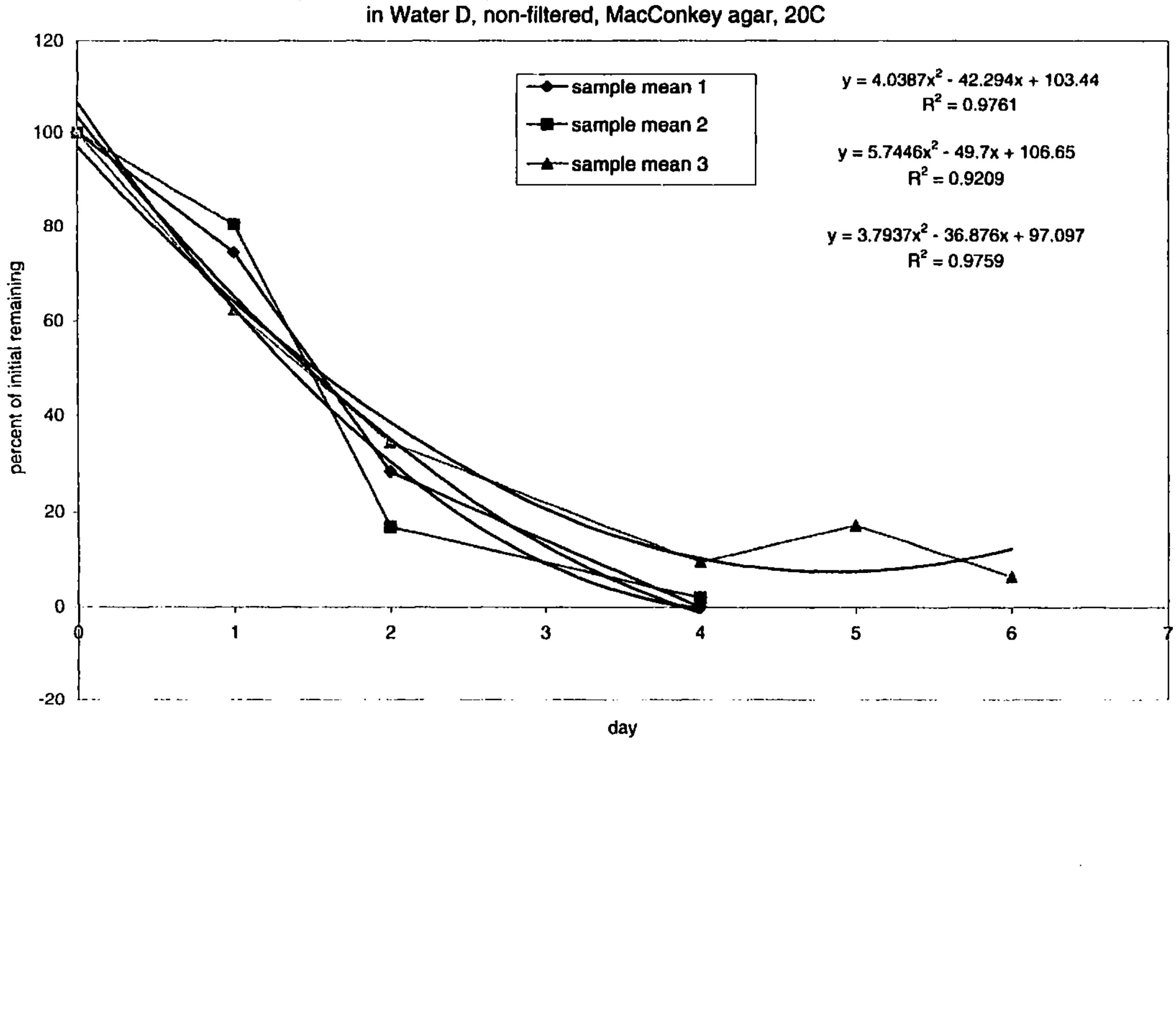
Appendix 82. Decline phase of E. coli O157:H7 waterborne isolate, in Water D, non-filtered, MacConkey agar, 10C



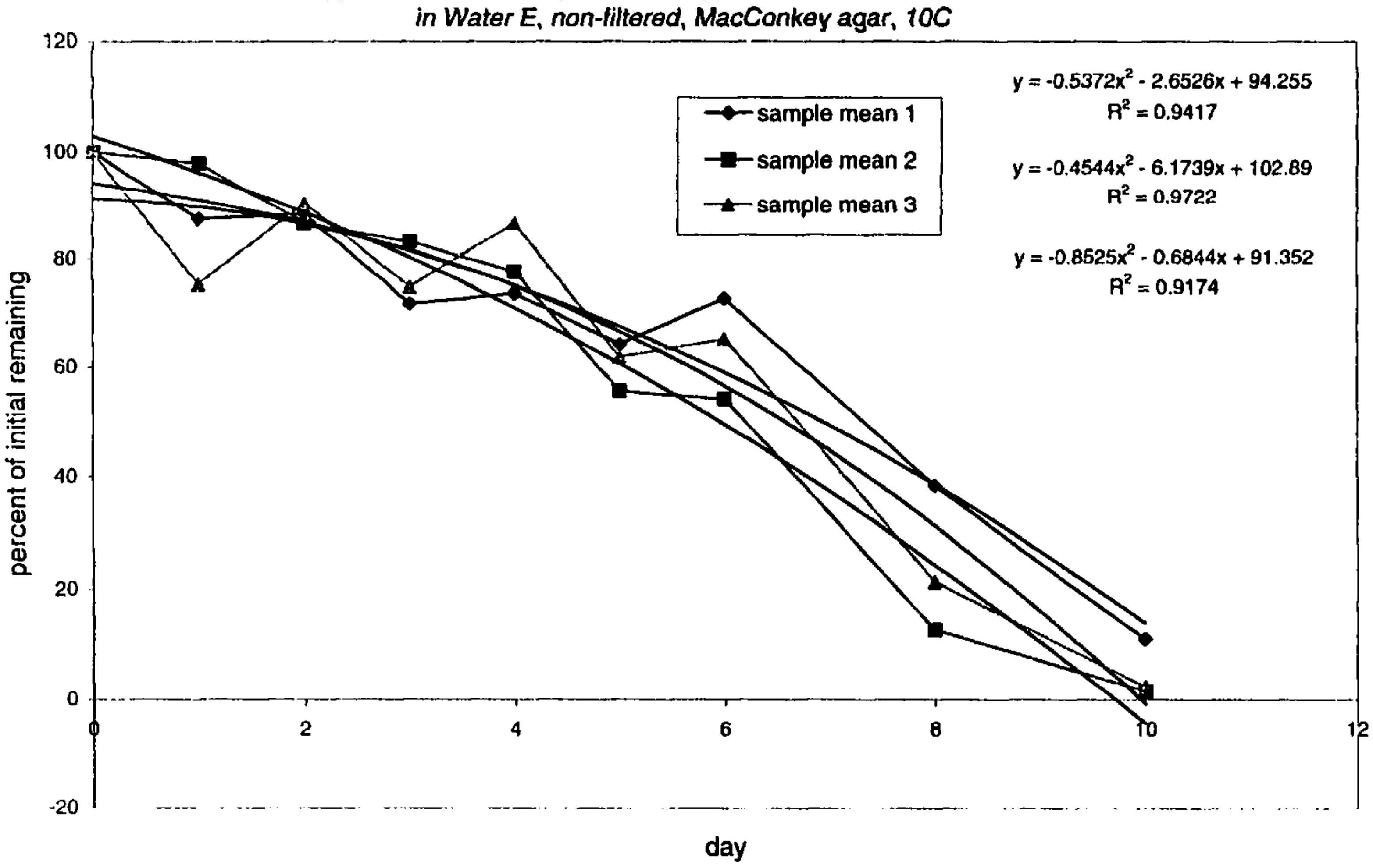
Appendix 83. Decline phase of S. typhimurium waterborne isolate, in Water D, non-filtered, MacConkey agar, 20C



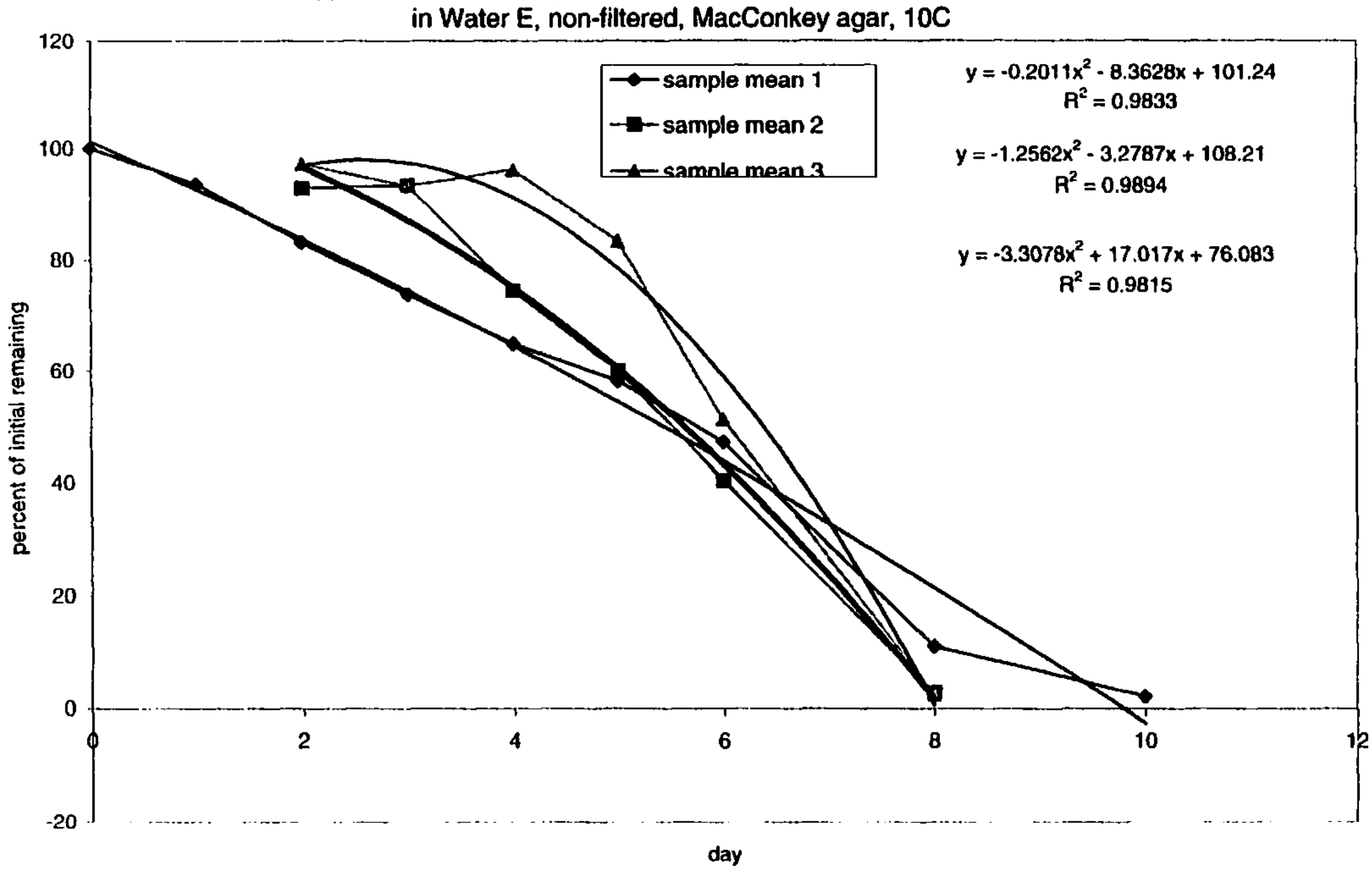
Appendix 84. Decline phase of E. coli O157:H7 waterborne isolate, in Water D, non-filtered, MacConkey agar, 20C



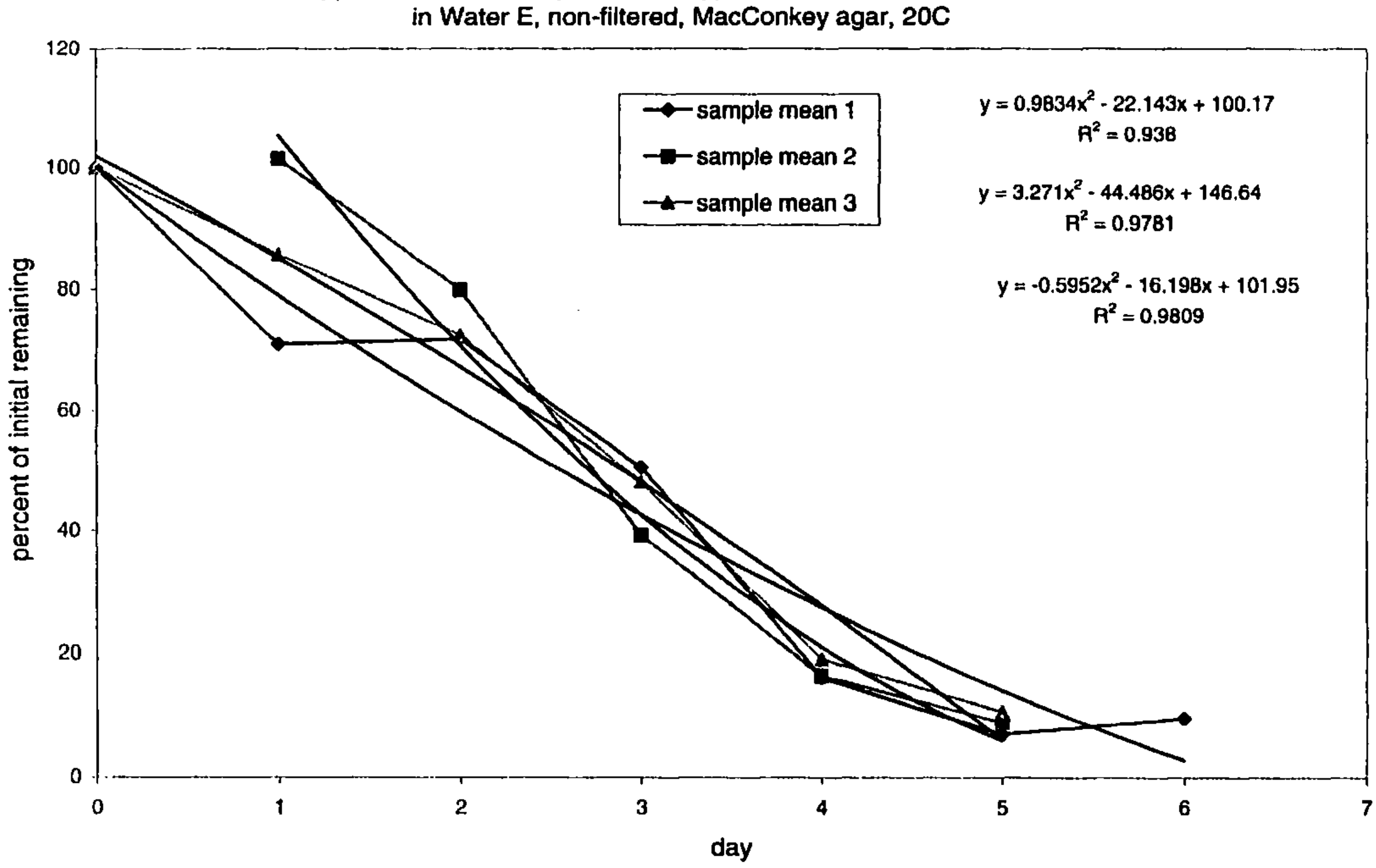
Appendix 85. Decline phase of S. typhimurium waterborne isolate,
in Water E, non-filtered, MacConkey agar, 10C



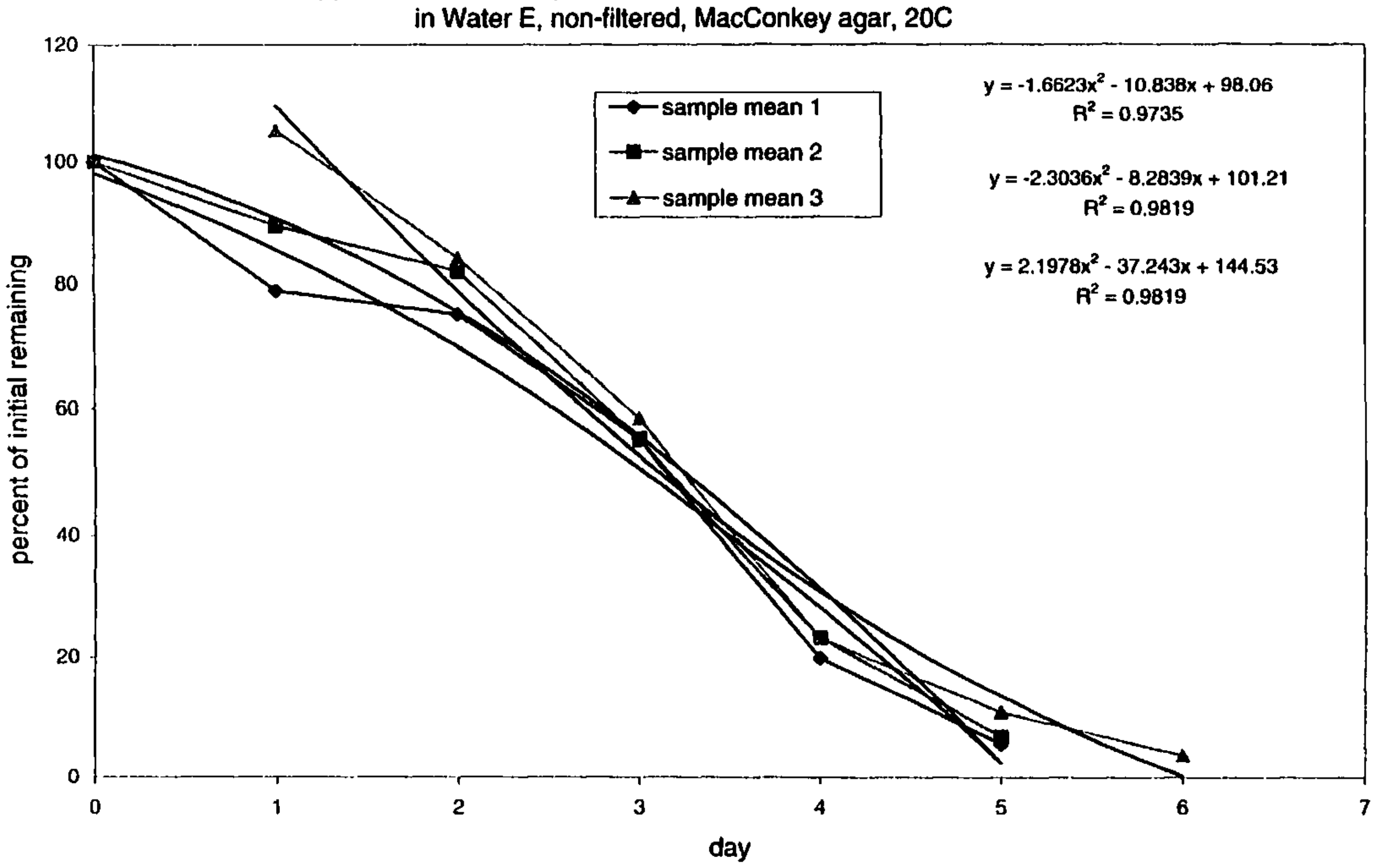
Appendix 86. Decline phase of E. coli O157:H7 waterborne isolate, in Water E, non-filtered, MacConkey agar, 10C



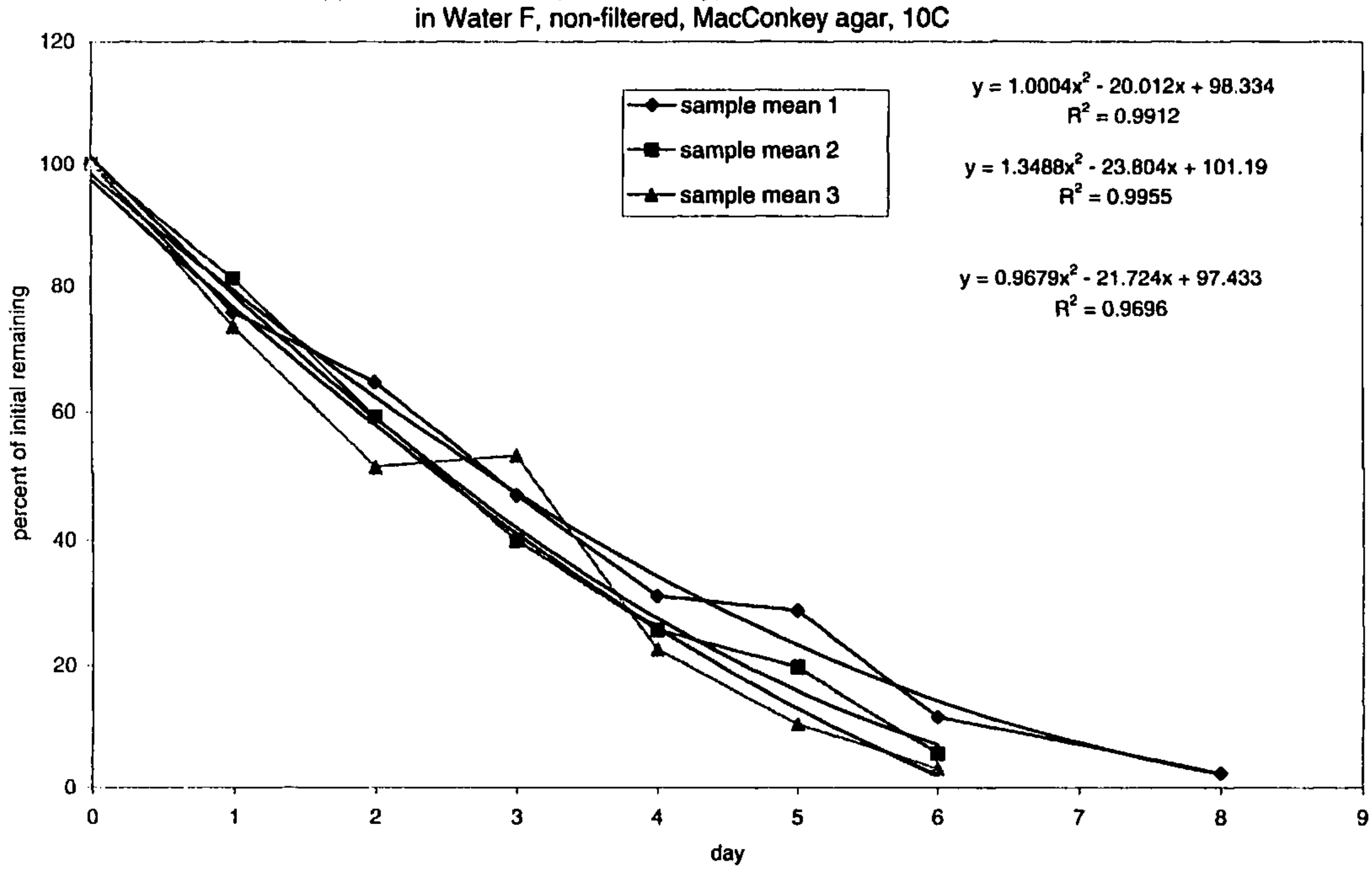
Appendix 87. Decline phase of S. typhimurium waterborne isolate, in Water E, non-filtered, MacConkey agar, 20C



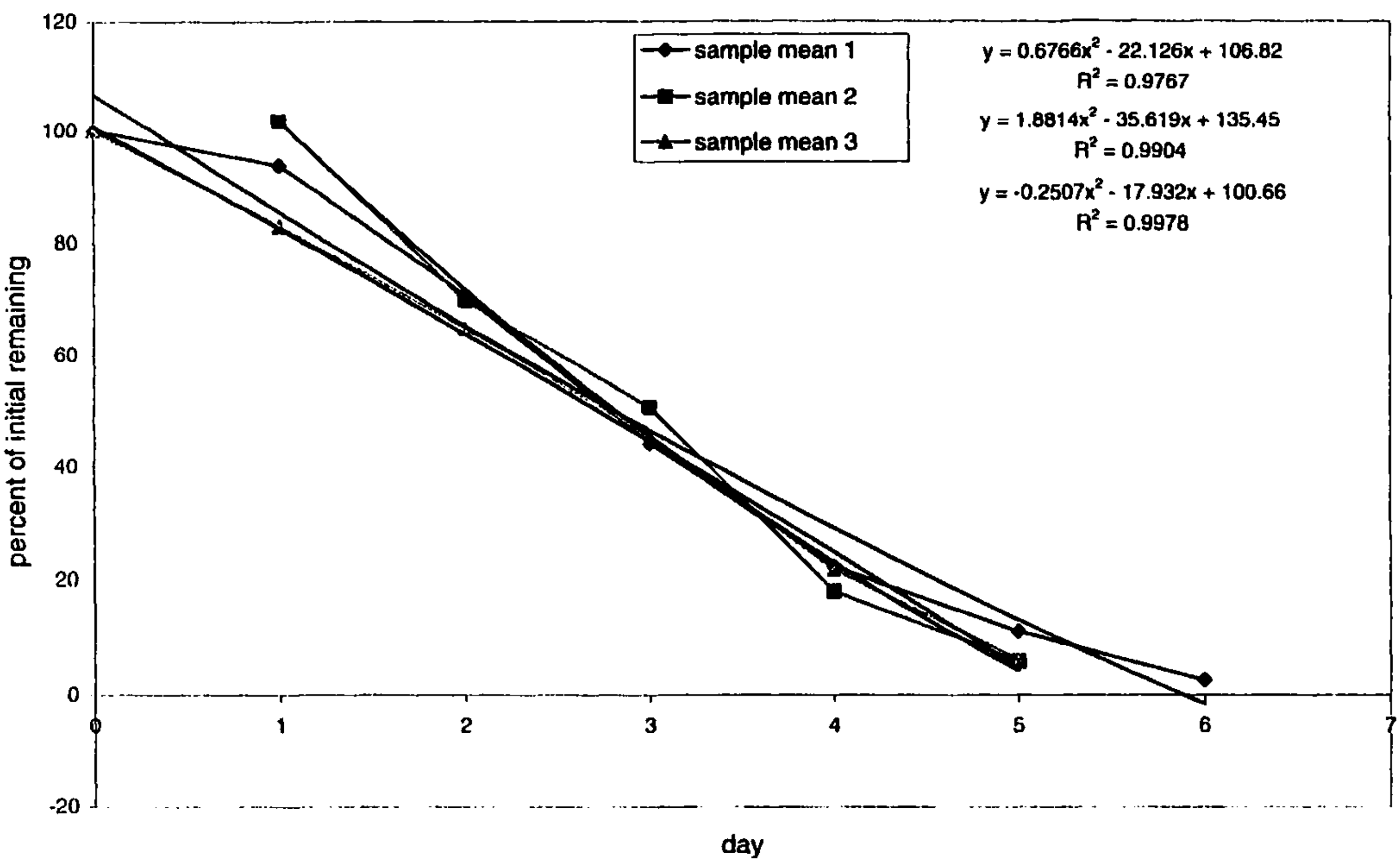
Appendix 88. Decline phase of E. coli O157:H7 waterborne isolate, in Water E, non-filtered, MacConkey agar, 20C



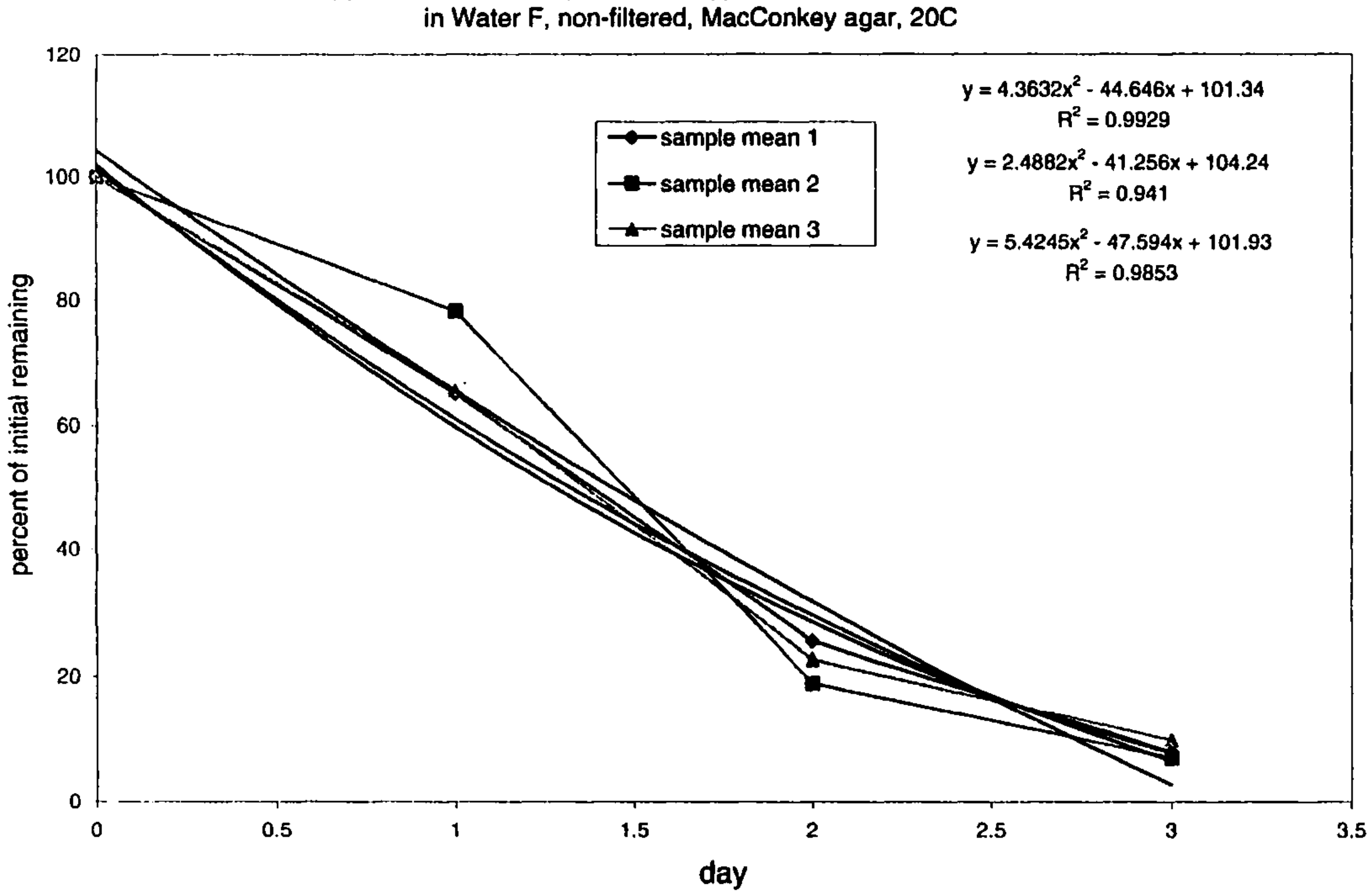
Appendix 89. Decline phase of S. typhimurium waterborne isolate, in Water F, non-filtered, MacConkey agar, 10C



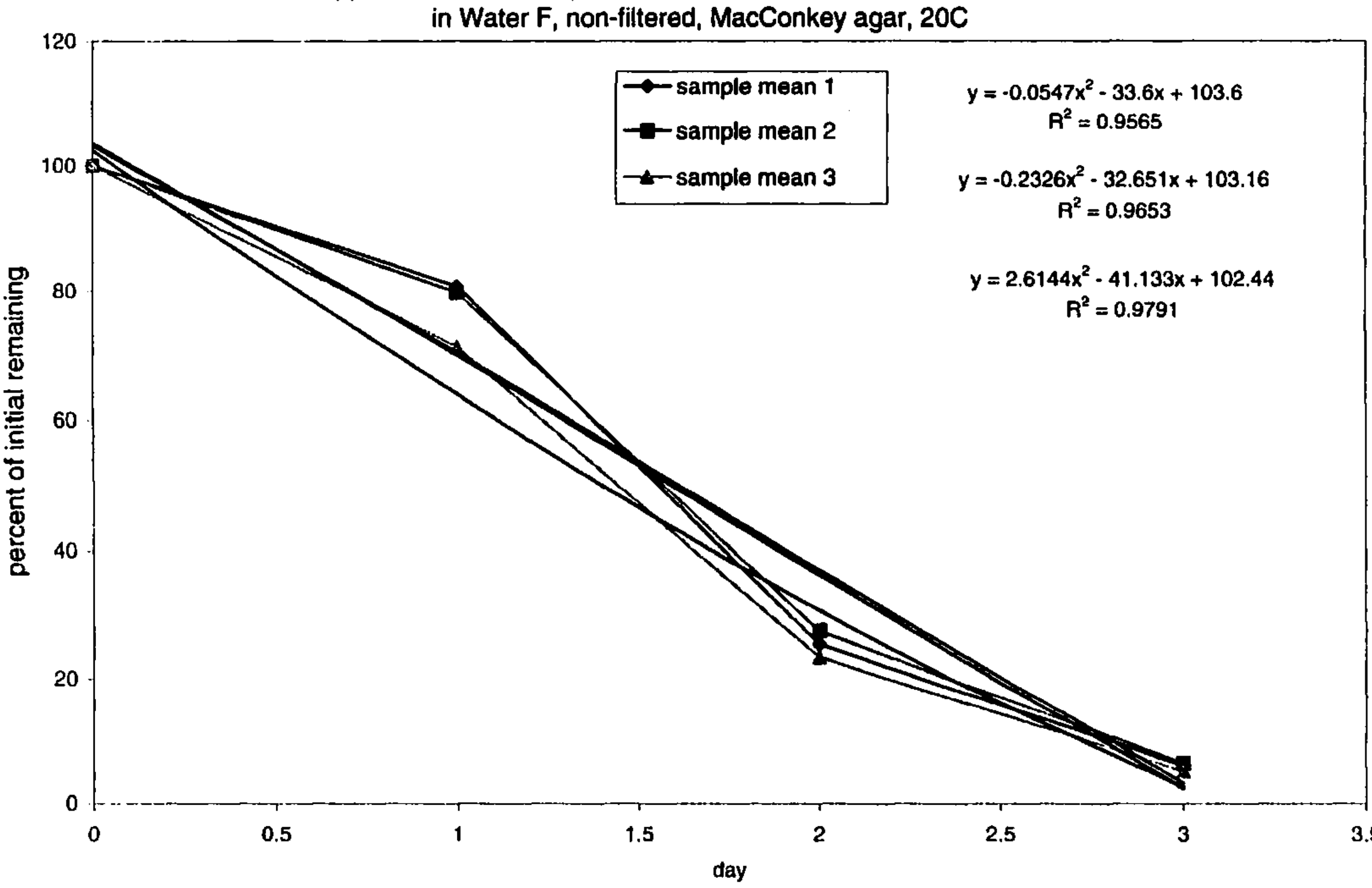
Appendix 90. Decline phase of E. coli O157:H7 waterborne isolate, in Water F non-filtered, MacConkey agar, 10C



Appendix 91. Decline phase of S. typhimurium waterborne isolate, in Water F, non-filtered, MacConkey agar, 20C



Appendix 92. Decline phase of E. coli O157:H7 waterborne isolate, in Water F, non-filtered, MacConkey agar, 20C



Appendix 93 Data of Survival Study of Pathogens in Waters A and B, on TSA

Water label	Sample	Autochthonous conc.: LB agar (CFU/100ul)	Turbidity (OD600)	Filtration (yes/no)	Temperature (C)	Species: S.typhimurium or E.coli	Source: water or other	Initial concentration (CFU/100ul)	Time to 100% of initial (d)	Time to 6.5%(non-filt) or 30%(filt) of initial (d)	polynomial coefficient a_1	Time to 0.1% of initial (d)	Duration of decline phase (d)	Duration of tail phase (d)	Acceleration (%/d^2)	Presence of shoulder phase
A	1	0	NA	n	10	S	W	192.00	0.00	12.29	-0.6817	12.99	12.29	0.70	-1.36	A
A	2	0	NA	n	10	S	W	192.00	0.00	8.85	-1.8180	12.23	8.85	3.38	-3.64	A
A	3	0	NA	n	10	S	W	192.00	0.00	12.24	-0.7334	16.23	12.24	4.00	-1.47	A
A	4	0	NA	n	10	S	W	192.00	0.00	8.92	-1.6380	12.62	8.92	3.69	-3.28	A
A	1	0	NA	n	20	S	W	192.00	0.00	10.56	-0.2576	16.62	10.56	6.06	-0.52	A
A	2	0	NA	n	20	S	W	192.00	0.00	10.79	-0.1768	20.85	10.79	10.06	-0.35	A
A	3	0	NA	n	20	S	W	192.00	0.00	8.36	-0.6467	28.78	8.36	20.41	-1.29	A
A	4	0	NA	n	20	S	W	192.00	0.00	11.70	-0.2354	20.85	11.70	9.14	-0.47	A
A	1	0	NA	y	10	S	W	143.00	5.33	27.36	0.0422	79.10	22.02	51.74	0.08	P
A	2	0	NA	y	10	S	W	143.00	9.06	31.22	0.0422	72.10	22.16	40.88	0.08	P
A	3	0	NA	y	10	S	W	143.00	6.78	33.84	0.0884	80.06	27.06	46.22	0.18	P
A	4	0	NA	y	10	S	W	143.00	5.69	34.82	0.0084	86.10	29.13	51.28	0.02	P
A	1	0	NA	y	20	S	W	143.00	12.07	52.03	-0.0024		39.96		0.00	P
A	2	0	NA	y	20	S	W	143.00	0.00	41.53	0.0027	87.50	41.53	45.97	0.01	A
A	3	0	NA	y	20	S	W	143.00	9.09	34.70	0.1336	93.00	25.61	58.29	0.27	P
A	4	0	NA	y	20	S	W	143.00	4.16	44.82	0.0080	80.67	40.66	35.85	0.02	P
A	1	0	NA	n	10	S	O	132.00	6.08	14.53	-0.5619	17.94	8.45	3.41	-1.12	P
A	2	0	NA	n	10	S	O	132.00	0.00	27.89	0.0350	32.92	27.89	5.03	0.07	A
A	3	0	NA	n	10	S	O	132.00	5.79	14.77	-0.7955	18.88	8.98	4.12	-1.59	P
A	4	0	NA	n	10	S	O	132.00	3.94	14.85	-0.7045	18.91	10.91	4.06	-1.41	P
A	1	0	NA	n	20	S	O	132.00	1.42	13.20	0.2293	17.94	11.78	4.74	0.46	P

Appendix 93 Data of Survival Study of Pathogens in Waters A and B, on TSA

Water label	Sample	Autochthonous conc.: LB agar (CFU/100ul)	Turbidity (OD600)	Filtration (yes/no)	Temperature (C)	Species: *S.typhimurium* or *E.coli*	Source: water or other	Initial concentration (CFU/100ul)	Time to 100% of initial (d)	Time to 6.5%(non-fit) or 30%(fit) of initial (d)	polynomial coefficient a_1	Time to 0.1% of initial (d)	Duration of decline phase (d)	Duration of tail phase (d)	Acceleration (%/d^2)	Presence of shoulder phase
A	2	0	NA	n	20	S	O	132.00	0.00	13.18	0.2034	18.47	13.18	5.29	0.41	A
A	3	0	NA	n	20	S	O	132.00	3.03	9.30	-0.5840	17.94	6.27	8.65	-1.17	P
A	4	0	NA	n	20	S	O	132.00	0.00	13.68	0.2972	18.47	13.68	4.79	0.59	A
A	1	0	NA	y	10	S	O	131.50	0.00	25.06	-0.0760		25.06		-0.15	A
A	2	0	NA	y	10	S	O	131.50	1.77	27.59	-0.1022	126.53	25.82	98.94	-0.20	P
A	3	0	NA	y	10	S	O	131.50	5.08	27.47	-0.1076	114.28	22.39	86.82	-0.22	P
A	4	0	NA	y	10	S	O	131.50	2.53	25.53	0.0021	114.28	23.00	88.76	0.00	P
A	1	0	NA	y	20	S	O	131.50	0.00	4.53	-2.8340	143.47	4.53	138.94	-5.67	A
A	2	0	NA	y	20	S	O	131.50	1.56	5.93	-1.3308		4.37		-2.66	P
A	3	0	NA	y	20	S	O	131.50	0.00	9.39	0.4959		9.39		0.99	A
A	4	0	NA	y	20	S	O	131.50	0.00	4.71	-4.4166		4.71		-8.83	A
A	1	0	NA	n	10	E	W	119.00	0.00	15.07	-0.2261	27.60	15.07	12.54	-0.45	A
A	2	0	NA	n	10	E	W	119.00	0.00	17.35	-0.1179	22.76	17.35	5.42	-0.24	A
A	3	0	NA	n	10	E	W	119.00	0.00	12.90	-0.2729	13.98	12.90	1.09	-0.55	A
A	4	0	NA	n	10	E	W	119.00	0.00	12.34	-0.6097	21.81	12.34	9.47	-1.22	A
A	1	0	NA	n	20	E	W	119.00	0.00	15.84	-0.0672	31.81	15.84	15.97	-0.13	A
A	2	0	NA	n	20	E	W	119.00	0.00	9.60	-0.9222	21.81	9.60	12.21	-1.84	A
A	3	0	NA	n	20	E	W	119.00	0.00	10.77	-0.5311	21.81	10.77	11.05	-1.06	A
A	4	0	NA	n	20	E	W	119.00	0.00	10.94	-0.2142	21.81	10.94	10.87	-0.43	A
A	1	0	NA	y	10	E	W	94.00	2.27	22.56	0.1172		20.29		0.23	P
A	2	0	NA	y	10	E	W	94.00	1.15	17.54	-0.0024	106.68	16.39	89.14	0.00	P

Appendix 93 Data of Survival Study of Pathogens in Waters A and B, on TSA

Water label	Sample	Autochthonous conc.: LB agar (CFU/100ul)	Turbidity (OD600)	Filtration (yes/no)	Temperature (C)	Species: *S.typhimurium* or *E.coli*	Source: water or other	Initial concentration (CFU/100ul)	Time to 100% of initial (d)	Time to 6.5%(non-fit) or 30%(fit) of initial (d)	polynomial coefficient a_1	Time to 0.1% of initial (d)	Duration of decline phase (d)	Duration of tail phase (d)	Acceleration (%/d^2)	Presence of shoulder phase
A	3	0	NA	y	10	E	W	94.00	1.35	94.21	0.0062		92.86		0.01	P
A	4	0	NA	y	10	E	W	94.00	2.37	46.94	0.0526	120.68	44.57	73.75	0.11	P
A	1	0	NA	y	20	E	W	94.00	0.00	63.27	0.0075	107.34	63.27	44.07	0.02	A
A	2	0	NA	y	20	E	W	94.00	0.00	7.27	1.8490	92.68	7.27	85.42	3.70	A
A	3	0	NA	y	20	E	W	94.00	0.00	6.87	1.5862	113.68	6.87	106.81	3.17	A
A	4	0	NA	y	20	E	W	94.00	0.00	16.23	0.1815	113.68	16.23	97.46	0.36	A
A	1	0	NA	n	10	E	O	109.00	3.84	13.69	-0.7408	17.93	9.85	4.25	-1.48	P
A	2	0	NA	n	10	E	O	109.00	2.44	11.80	-1.4889	17.90	9.36	6.10	-2.98	P
A	3	0	NA	n	10	E	O	109.00	0.00	12.88	-1.1909	20.35	12.88	7.47	-2.38	A
A	4	0	NA	n	10	E	O	109.00	3.54	15.24	0.2184	17.96	11.71	2.71	0.44	P
A	1	0	NA	n	20	E	O	109.00	0.00	15.21	0.5843	31.91	15.21	16.70	1.17	A
A	2	0	NA	n	20	E	O	109.00	0.00	16.37	0.3080		16.37		0.62	A
A	3	0	NA	n	20	E	O	109.00	0.00	12.99	0.5830	31.91	12.99	18.92	1.17	A
A	4	0	NA	n	20	E	O	109.00	0.00	16.55	0.2619	32.46	16.55	15.91	0.52	A
A	1	0	NA	y	10	E	O	123.00	0.00	25.77	0.0809		25.77		0.16	A
A	2	0	NA	y	10	E	O	123.00	0.00	40.61	0.0711		40.61		0.14	A
A	3	0	NA	y	10	E	O	123.00	3.17	27.19	0.1195		24.02		0.24	P
A	4	0	NA	y	10	E	O	123.00	0.00	26.39	0.0458		26.39		0.09	A
A	1	0	NA	y	20	E	O	123.00	0.00	71.13	-0.0125	113.85	71.13	42.72	-0.03	A
A	2	0	NA	y	20	E	O	123.00	0.00	66.25	0.0009	113.85	66.25	47.60	0.00	A
A	3	0	NA	y	20	E	O	123.00	0.00	67.76	-0.0093	113.28	67.76	45.52	-0.02	A

Appendix 93 Data of Survival Study of Pathogens in Waters A and B, on TSA

Water label	Sample	Autochthonous conc.: LB agar (CFU/100ul)	Turbidity (OD600)	Filtration (yes/no)	Temperature (C)	Species: *S.typhimurium* or *E.coli*	Source: water or other	Initial concentration (CFU/100ul)	Time to 100% of initial (d)	Time to 6.5%(non-fit) or 30%(fit) of initial (d)	polynomial coefficient a_1	Time to 0.1% of initial (d)	Duration of decline phase (d)	Duration of tail phase (d)	Acceleration (%/d^2)	Presence of shoulder phase
A	4	0	NA	y	20	E	O	123.00	0.00	75.51	-0.0187	125.56	75.51	50.05	-0.04	A
B	1	55.5	0.0095	n	10	S	W	121.50	1.41	7.95	-0.2338	11.77	6.54	3.82	-0.47	P
B	2	55.5	0.0095	n	10	S	W	122.00	0.00	7.29	-0.2451	11.77	7.29	4.48	-0.49	A
B	3	55.5	0.0095	n	10	S	W	96.00	0.00	7.05	-0.1441	11.65	7.05	4.60	-0.29	A
B	1	55.5	0.0095	n	20	S	W	73.50	1.04	3.65		4.00	2.61	0.34		P
B	2	55.5	0.0095	n	20	S	W	64.50	0.00	3.71	-0.7206	7.78	3.71	4.07	-1.44	A
B	3	55.5	0.0095	n	20	S	W	83.50	1.02	3.75		4.85	2.73	1.10		P
B	1	55.5	0.0095	y	10	S	W	119.00	0.00	27.09	0.1337		27.09		0.27	A
B	2	55.5	0.0095	y	10	S	W	116.50	1.42	31.25	0.0678		29.83		0.14	P
B	3	55.5	0.0095	y	10	S	W	120.50	0.00	41.95	0.0410		41.95		0.08	A
B	1	55.5	0.0095	y	20	S	W	129.00	22.61	76.63	0.0019		54.02		0.00	P
B	2	55.5	0.0095	y	20	S	W	115.00	26.88	72.57	-0.0076		45.69		-0.02	P
B	3	55.5	0.0095	y	20	S	W	130.00	75.96		-0.0218					P
B	1	55.5	0.0095	n	10	E	W	50.00	0.00	9.13	0.4287	13.84	9.13	4.71	0.86	A
B	2	55.5	0.0095	n	10	E	W	36.00	0.00	8.66	-0.9857	9.99	8.66	1.32	-1.97	A
B	3	55.5	0.0095	n	10	E	W	27.00	0.00	7.95	-0.2315	16.90	7.95	8.95	-0.46	A
B	1	55.5	0.0095	n	20	E	W	44.50	0.00	3.70	-0.2273	4.00	3.70	0.29	-0.45	A
B	2	55.5	0.0095	n	20	E	W	38.00	1.06	3.81		7.97	2.75	4.16		P
B	3	55.5	0.0095	n	20	E	W	44.00	0.00	3.69	0.7490	4.93	3.69	1.25	1.50	A
B	1	55.5	0.0095	y	10	E	W	51.50	1.25	15.98	0.4651		14.73		0.93	P
B	2	55.5	0.0095	y	10	E	W	56.00	3.57	22.22	0.0941		18.65		0.19	P

Appendix 93 Data of Survival Study of Pathogens in Waters A and B, on TSA

Water label	Sample	Autochthonous conc.: LB agar (CFU/100ul)	Turbidity (OD600)	Filtration (yes/no)	Temperature (C)	Species: *S.typhimurium* or *E.coli*	Source: water or other	Initial concentration (CFU/100ul)	Time to 100% of initial (d)	Time to 6.5%(non-filt) or 30%(filt) of initial (d)	polynomial coefficient a_1	Time to 0.1% of initial (d)	Duration of decline phase (d)	Duration of tail phase (d)	Acceleration (%/d^2)	Presence of shoulder phase
B	3	55.5	0.0095	y	10	E	W	89.00	0.00	25.46	0.2385		25.46		0.48	A
B	1	55.5	0.0095	y	20	E	W	66.50	0.00	8.01	1.3453		8.01		2.69	A
B	2	55.5	0.0095	y	20	E	W	58.50	51.20		-0.0048					P
B	3	55.5	0.0095	y	20	E	W	45.00	38.14	72.53	-0.0360		34.39		-0.07	P

Appendix 94 Data of Survival Study of Pathogens in Waters B through F, on MacConkey Agar

Site code	Sample	Autochthonous conc.: LB agar (CFU/100ul)	Turbidity (OD600)	Filtration yes/no)	Temperature	Species: *S.typhimurium* or *E.coli*	Source: water or other	Initial concentration (CFU/100ul)	Time to 100% of initial (days)	Time to 6.5% of initial conc. (days)	polynomial coefficient a_1	Duration of decline phase (d)	Duration of tail phase (days)	Time to 0.1% of initial (days)	Acceleration (%/d^2)	Presence of shoulder phase
B	1	55.5	0.0095	n	10	S	W	104.86	1.42	7.60	0.7949	6.18	2.30	9.90	1.59	P
B	2	55.5	0.0095	n	10	S	W	105.37	0.00	6.33	-1.1313	6.33	1.65	7.98	-2.26	A
B	3	55.5	0.0095	n	10	S	W	74.75	0.00	5.92	-1.2439	5.92	2.04	7.97	-2.49	A
B	1	55.5	0.0095	n	10	E	W	29.18	0.00	8.10	0.8587	8.10	1.88	9.98	1.72	A
B	2	55.5	0.0095	n	10	E	W	19.48	0.00	7.62	-0.9616	7.62	4.34	11.96	-1.92	A
B	3	55.5	0.0095	n	10	E	W	13.75	1.99	8.21	0.6946	6.22	1.78	9.99	1.39	P
B	1	55.5	0.0095	n	20	S	W	50.99	1.05	3.47		2.42	0.53	3.99		P
B	2	55.5	0.0095	n	20	S	W	42.20	0.00	3.59	1.9159	3.59	2.91	6.50	3.83	A
B	3	55.5	0.0095	n	20	S	W	61.16	1.02	3.62		2.60	0.37	4.00		P
B	1	55.5	0.0095	n	20	E	W	25.26	0.00	3.65	1.2929	3.65	0.35	4.00	2.59	A
B	2	55.5	0.0095	n	20	E	W	20.91	1.05	3.76		2.71	6.22	9.98		P
B	3	55.5	0.0095	n	20	E	W	24.94	0.00	3.56	2.0653	3.56	1.30	4.86	4.13	A
C	1	91.5	0.0215	n	10	S	W	134.50	0.00	7.97	-0.0903	7.97	8.70	16.66	-0.18	A
C	2	91.5	0.0215	n	10	S	W	127.50	0.00	8.25	-0.0915	8.25	8.44	16.69	-0.18	A
C	3	91.5	0.0215	n	10	S	W	136.00	0.00	7.96	0.5788	7.96	8.81	16.77	1.16	A
C	1	91.5	0.0215	n	10	E	W	65.50	0.00	9.62	0.4870	9.62	10.19	19.81	0.97	A
C	2	91.5	0.0215	n	10	E	W	50.50	0.00	10.10	-0.6064	10.10	12.84	22.94	-1.21	A
C	3	91.5	0.0215	n	10	E	W	55.50	0.00	9.84	0.4465	9.84	7.09	16.93	0.89	A
C	1	91.5	0.0215	n	20	S	W	125.00	0.00	3.67	4.0266	3.67	1.80	5.47	8.05	A
C	2	91.5	0.0215	n	20	S	W	128.50	0.00	3.67	2.0344	3.67	1.55	5.23	4.07	A
C	3	91.5	0.0215	n	20	S	W	118.50	0.00	3.65	1.8030	3.65	2.98	6.63	3.61	A
C	1	91.5	0.0215	n	20	E	W	53.50	0.00	3.86	-5.1756	3.86	3.98	7.84	-10.35	A
C	2	91.5	0.0215	n	20	E	W	65.00	0.00	4.00	1.5033	4.00	7.88	11.88	3.01	A

Appendix 94 Data of Survival Study of Pathogens in Waters B through F, on MacConkey Agar

Site code	Sample	Autochthonous conc.: LB agar (CFU/100ul)	Turbidity (OD600)	Filtration yes/no)	Temperature	Species: *S.typhimurium* or *E.coli*	Source: water or other	Initial concentration (CFU/100ul)	Time to 100% of initial (days)	Time to 6.5% of initial conc. (days)	polynomial coefficient a_1	Duration of decline phase (d)	Duration of tail phase (days)	Time to 0.1% of initial (days)	Acceleration (%/d^2)	Presence of shoulder phase
C	3	91.5	0.0215	n	20	E	W	50.50	0.00	3.98	-5.8536	3.98	1.97	5.95	-11.71	A
D	1	12	0.0030	n	10	S	W	106.00	0.00	7.17	0.1560	7.17	15.64	22.81	0.31	A
D	2	12	0.0030	n	10	S	W	600.46	0.00	5.54	0.9477	5.54	10.86	16.40	1.90	A
D	3	12	0.0030	n	10	S	W	88.50	0.00	6.69	-0.9844	6.69	9.74	16.42	-1.97	A
D	1	12	0.0030	n	10	E	W	60.00	1.27	8.50	1.9036	7.23	8.33	16.84	3.81	P
D	2	12	0.0030	n	10	E	W	48.00	0.00	6.93	-0.0018	6.93	3.04	9.96	0.00	A
D	3	12	0.0030	n	10	E	W	65.00	0.00	5.56	0.3162	5.56	0.44	5.99	0.63	A
D	1	12	0.0030	n	20	S	W	126.50	0.00	3.30	8.1336	3.30	2.16	5.46	16.27	A
D	2	12	0.0030	n	20	S	W	127.50	0.00	3.40	6.3384	3.40	19.29	22.69	12.68	A
D	3	12	0.0030	n	20	S	W	127.00	0.00	3.67	2.9237	3.67	1.32	4.99	5.85	A
D	1	12	0.0030	n	20	E	W	53.50	0.00	3.54	4.0387	3.54	0.46	4.00	8.08	A
D	2	12	0.0030	n	20	E	W	49.00	0.00	3.40	5.7446	3.40	2.44	5.83	11.49	A
D	3	12	0.0030	n	20	E	W	51.50	0.00	6.00	3.7937	6.00	16.94	22.94	7.59	A
E	1	77	0.0020	n	10	S	W	108.00	0.00	10.95	-0.5372	10.95	13.25	24.20	-1.07	A
E	2	77	0.0020	n	10	S	W	98.00	0.00	9.11	-0.4544	9.11	7.62	16.73	-0.91	A
E	3	77	0.0020	n	10	S	W	105.00	0.00	9.57	-0.8525	9.57	6.85	16.42	-1.71	A
E	1	77	0.0020	n	10	E	W	266.50	0.00	9.01	-0.2011	9.01	7.21	16.22	-0.40	A
E	2	77	0.0020	n	10	E	W	226.50	1.50	7.81	-1.2562	6.31	5.31	13.12	-2.51	P
E	3	77	0.0020	n	10	E	W	210.00	1.79	7.83	-3.3078	6.05	3.35	11.18	-6.62	P
E	1	77	0.0020	n	20	S	W	115.00	0.00	6.78	0.9834	6.78	21.36	28.14	1.97	A
E	2	77	0.0020	n	20	S	W	107.00	1.07	5.46	3.2710	4.39	11.24	16.71	6.54	P
E	3	77	0.0020	n	20	S	W	112.50	0.00	5.41	-0.5952	5.41	11.28	16.69	-1.19	A
E	1	77	0.0020	n	20	E	W	260.50	0.00	4.92	-1.6623	4.92	11.32	16.24	-3.32	A

Appendix 94 Data of Survival Study of Pathogens in Waters B through F, on MacConkey Agar

Site code	Sample	Autochthonous conc.: LB agar (CFU/100ul)	Turbidity (OD600)	Filtration yes/no)	Temperature	Species: *S.typhimurium* or *E.coli*	Source: water or other	Initial concentration (CFU/100ul)	Time to 100% of initial (days)	Time to 6.5% of initial conc. (days)	polynomial coefficient a_1	Duration of decline phase (d)	Duration of tail phase (days)	Time to 0.1% of initial (days)	Acceleration (%/d^2)	Presence of shoulder phase
E	2	77	0.0020	n	20	E	W	231.00	0.00	5.00	-2.3036	5.00	8.10	13.10	-4.61	A
E	3	77	0.0020	n	20	E	W	227.50	1.25	5.58	2.1978	4.33	5.98	11.56	4.40	P
F	1	79	0.0255	n	10	S	W	107.50	0.00	7.10	1.0004	7.10	2.82	9.92	2.00	A
F	2	79	0.0255	n	10	S	W	109.00	0.00	5.93	1.3488	5.93	7.67	13.60	2.70	A
F	3	79	0.0255	n	10	S	W	115.00	0.00	5.53	0.9679	5.53	4.26	9.79	1.94	A
F	1	79	0.0255	n	10	E	W	230.50	0.00	5.54	0.6766	5.54	5.56	11.10	1.35	A
F	2	79	0.0255	n	10	E	W	224.00	1.06	4.96	1.8814	3.90	4.17	9.13	3.76	P
F	3	79	0.0255	n	10	E	W	231.50	0.00	4.98	-0.2507	4.98	4.11	9.10	-0.50	A
F	1	79	0.0255	n	20	S	W	106.00	0.00	3.20	4.3632	3.20	8.60	11.81	8.73	A
F	2	79	0.0255	n	20	S	W	105.50	0.00	3.12	2.4882	3.12	13.59	16.71	4.98	A
F	3	79	0.0255	n	20	S	W	106.00	0.00	3.45	5.4245	3.45	13.26	16.71	10.85	A
F	1	79	0.0255	n	20	E	W	228.50	0.00	2.97	-0.0547	2.97	12.69	15.66	-0.11	A
F	2	79	0.0255	n	20	E	W	215.00	0.00	2.99	-0.2328	2.99	4.17	7.16	-0.47	A
F	3	79	0.0255	n	20	E	W	229.50	0.00	2.92	2.6144	2.92	6.64	9.56	5.23	A

Appendix 95-1. Statistical measures of time to 0.1% of C_0, $t_{0.1}$ for samples Waters A and B. counts on TSA

	Water label		Temperature		Filtration		Species		Isolate origin	
	A	B	10^{0}C	20^{0}C	Filtered	Non-filtered	*E. coli* O157:H7	*S. typhi-murium*	Water	Other
n	52	9	31	30	21	40	30	31	38	23
Mean	54.90	10.29	41.25	55.62	104.68	18.73	50.83	45.89	43.43	56.39
Standard deviation	43.28	4.14	39.91	45.48	18.83	7.31	44.88	41.69	39.08	48.62
Minimum	12.23	4.00	9.99	4.00	72.10	4.00	4.00	7.78	4.00	17.90
Maximum	143.47	16.90	126.53	143.47	143.47	32.92	125.56	143.47	120.68	143.47

Appendix 95-2. Statistical measures of duration of decline phase., □$t_{decline}$, for samples Waters A and B, counts on TSA

	Water label		Temperature		Filtration		Species		Isolate origin	
	A	B	10^0C	20^0C	Filtered	Non-filtered	*E. coli* O157:H7	*S. typhi-murium*	Water	Other
n	52	9	31	30	21	40	30	31	38	23
Mean	21.10	6.41	15.46	22.53	10.90	34.26	22.06	15.91	16.03	23.74
Standard deviation	18.02	2.18	8.78	22.89	22.41	4.31	22.44	10.16	12.92	22.61
Minimum	4.53	3.69	6.54	3.69	4.53	3.69	3.69	3.71	3.69	4.53
Maximum	75.51	9.13	44.57	71.82	75.51	27.89	75.51	41.53	63.27	75.51

Appendix 95-3. Statistical measures of duration of tail phase, □t_{tail}, for samples Waters A and B, counts on TSA

	Water label		Temperature		Filtration		Species		Isolate origin	
	A	B	10^0C	20^0C	Filtered	Non-filtered	*E. coli* O157:H7	*S. typhi-murium*	Water	Other
n	52	9	31	30	21	40	30	31	38	23
Mean	32.20	3.72	23.64	32.50	7.04	67.92	28.32	27.69	26.22	30.93
Standard deviation	34.73	2.58	32.17	35.02	5.12	28.07	32.25	35.42	30.80	38.37
Minimum	0.70	0.29	0.70	0.29	0.29	35.85	0.29	0.70	0.29	2.71
Maximum	138.9	8.95	98.94	138.9	20.41	138.9	106.8	138.9	106.8	138.9

Appendix 95-4. Statistical measures of acce;eration, A, for samples from Sites A and B, counts on TSA

	Water label		Temperature		Filtration		Species		Isolate origin	
	A	B	10^0C	20^0C	Filtered	Non-filtered	*E. coli* O157:H7	*S. typhi-murium*	Water	Other
n	52	9	31	30	21	40	30	31	38	23
Mean	-0.46	-0.36	-0.82	-0.05	0.09	-0.72	-0.09	-0.78	-0.36	-0.59
Standard deviation	1.47	1.05	1.12	1.58	1.67	1.18	1.41	1.34	1.34	1.54
Minimum	-5.67	-1.97	-3.64	-5.67	-5.67	-3.64	-2.98	-5.67	-3.64	-5.67
Maximum	3.70	1.50	0.86	3.70	3.70	1.50	3.70	0.59	3.70	1.17

Appendix 95-5. Statistical measures of time to 0.1% of C_0, $t_{0.1}$, for sample Waters B through F, MacConkey agar

	Water label					Temperature		Species	
	B	C	D	E	F	10^0C	20^0C	*E. coli* O157:H7	*Salmonella*
n	12	12	12	12	12	30	30	30	30
Mean	7.59	12.73	12.86	16.69	11.69	13.88	10.74	11.49	13.13
Standard deviation	2.85	6.30	7.62	4.96	3.24	4.80	6.54	5.13	6.58
Minimum	3.99	5.23	4.00	11.18	7.16	5.99	3.99	4.00	3.99
Maximum	11.96	22.94	22.94	28.14	16.71	24.20	28.14	22.94	28.14

Appendix 95-6. Statistical measures of duration of decline phase., $\Delta t_{decline}$, for sample Waters B through F, MacConkey agar

	Water label					Temperature		Species	
	B	C	D	E	F	10^0C	20^0C	*E. coli* O157:H7	*Salmonella*
n	12	12	12	12	12	30	30	30	30
Mean	4.91	6.38	5.20	6.82	4.30	7.24	3.81	5.49	5.55
Standard deviation	2.03	2.78	1.63	2.27	1.44	1.73	-0.99	2.21	2.27
Minimum	2.42	3.65	3.30	4.33	2.92	3.90	2.42	2.71	2.42
Maximum	8.10	10.10	7.23	10.95	7.10	10.95	6.78	10.10	10.95

Appendix 95-7. Statistical measures of duration of tail phase. □t_{tail}, for sample Waters B through F, MacConkey agar

	Water label					Temperature		Species	
	B	C	D	E	F	10^0C	20^0C	*E. coli* O157:H7	*Salmonella*
n	12	12	12	12	12	30	30	30	30
Mean	2.14	6.35	7.56	9.41	7.30	6.34	6.76	5.67	7.43
Standard deviation	1.71	3.76	6.90	4.76	3.91	3.86	6.00	4.08	5.72
Minimum	0.35	1.55	0.44	3.35	2.82	0.44	0.35	0.35	0.37
Maximum	6.22	12.84	19.29	21.36	13.59	15.64	21.36	16.94	21.36

Appendix 95-8. Statistical measures of acceleration *A* for sample Waters B through F, MacConkey agar

	Water label					Temperature		Species	
	B	C	D	E	F	10^0C	20^0C	*E. coli* O157:H7	*Salmonella*
n	12	12	12	12	12	30	30	30	30
Mean	0.72	-0.16	5.55	-0.79	3.37	0.07	3.41	0.58	2.90
Standard deviation	2.23	5.66	5.78	3.67	3.58	2.16	6.16	4.89	4.65
Minimum	-2.49	-11.71	-1.97	-6.62	-0.50	-6.62	-11.71	-11.71	-2.49
Maximum	4.13	8.05	16.27	6.54	10.85	3.81	16.27	11.49	16.27