NEW ZEALAND FOOD MONITORING PROGRAMME

FOOD SAFETY AND HYDROPONICALLY CULTIVATED VEGETABLES

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FOOD SAFETY AND HYDROPONICALLY CULTIVATED VEGETABLES

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- Andrew Hudson for the literature review and guidance.

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SUMMARY

This project examined three categories of hydroponically grown vegetables, from throughout New Zealand, for the presence of pathogens and indicator organisms. The three categories chosen (leafy vegetable, sprouts and herbs) may be eaten in an uncooked state and so present a potential vehicle of pathogenic organisms.

There are no recent data on the incidence of microbial hazards in hydroponically grown vegetables in New Zealand, but seed sprouts have been implicated in outbreaks overseas and for this reason a more intensive survey of this category was done. This involved a HACCP based questionnaire to evaluate the procedures used. A comparison of microbial results and the questionnaire responses was performed.

In total 291 samples, comprising 46 sprout samples from eight identified producers, a further 71 sprout samples from commercial retail outlets, 114 leafy vegetable samples, and 60 herb samples were tested.

No pathogens were found but *Escherichia coli* was isolated from 11.7% of the samples. This was broken down further to show 12.8 % of sprouts, 14% of leafy vegetables and 5% of herbs contained *E. coli*. This indicates that there is a small and variable potential risk of the three categories of hydroponically grown vegetables harbouring a pathogen.

The HACCP questionnaire results showed that there is considerable scope to increase the number of control points used in hydroponic vegetable production. Growers should be encouraged to use seed disinfection as the primary defence against contamination of their product as this has been identified as the most important control point. The implementation of HACCP based food safety programmes in hydroponic growing operations will minimise the risk of contamination.

RECOMMENDATIONS

Recommendation: Seed disinfection should be promoted as the primary defence against contamination of hydroponically cultivated vegetables.

Recommendation: Hydroponic vegetable growers should adopt HACCP based food safety programmes appropriate for their products.

INTRODUCTION

Hydroponics are defined as "the cultivation of plants, without using soil, by feeding them on chemical solutions". To replace soil, inert media (such as expanded clay, growool, Perlite and vermiculite) may be used. These contain no plant nutrients in themselves and nutrients are supplied in the water used to irrigate the crop. In media-less systems, no media are used except for an initial "starter cube".

This description may give the impression that pathogenic organisms which are normally associated with contamination by soil will not be a problem with hydroponically grown vegetables, but it should be noted that in, for example, seed sprout production the seed itself will have been grown conventionally and so may harbour soil organisms. However, it seems likely that contamination of vegetables grown hydroponically should be easier to prevent than with similar foods grown traditionally.

In New Zealand a number of hydroponically grown foods are produced. This project focused on lettuce and spinach (collectively termed "leafy vegetables" here), herbs, and seed sprouts. These products are in more intimate contact with the nutrient source than are, for example, tomatoes (although overhead irrigation does occur in some systems). All of these foods may be eaten in an uncooked state and so present a potential vehicle of pathogenic micro-organisms.

Of the hydroponically grown foods, seed sprouts have achieved recent prominence with several US regulatory authorities issuing advisories informing people who may be immunocompromised not to eat uncooked sprouts after a recent spate of outbreaks of *Salmonella* and *E. coli* O157:H7 in California. The literature data available (See Appendix I, Literature Review) are almost entirely related to seed sprouts because of the emergence of outbreaks associated with sprout consumption.

Despite the fact that vegetables are generally regarded as low risk foods it is clear that they can in fact harbour a range of pathogens. Given that these foods are not necessarily cooked before consumption they therefore pose a risk to the consumer.

Seed sprout production has been relatively well researched and hazards are quite well defined. However, no data were found which specifically addressed hydroponic vegetables other than bean sprouts. There are no recent data on the incidence of microbial hazards in these foods in New Zealand or on the adoption of HACCP systems by the industry.

PROJECT PLAN

- All producers of the three categories of hydroponically grown vegetables were identified.
- A HACCP questionnaire for the sprout producers was developed.
- Health Protection Officers arranged sampling dates with the identified producers. This was organised to allow for the sampling of different production runs where possible. It was also arranged in conjunction with the laboratories to ensure manageable numbers of samples arriving at any one time for analysis.
- A sampling plan for the collection of a suitable number of samples from the different categories was developed.
- Where appropriate the HACCP questionnaire was completed and sent to the project team for analysis.
- Samples submitted to the appropriate laboratory.
- Samples tested and reported.
- All results analysed and report prepared.

HACCP QUESTIONNAIRE

For sprout production a HACCP questionnaire (Appendix II) was developed from the flow chart (Figure 2, Appendix I) describing seed sprout production, and identifying points where potential hazards could occur (Table 12). Each of the originally identified eight sprout producers was assessed against this questionnaire on the first of the visits to the premises. During the commercial retail sampling two further producers were identified, but were not included in the HACCP analysis.

SAMPLING AND MICROBIAL ANALYSIS

Sampling Plan

Hydroponic vegetable samples comprising:

46 sprout samples
114 leafy vegetables
60 herb samples

were taken from hydroponic vegetable producers' premises throughout New Zealand between October 1998 and May 1999. A further 71 sprout samples were obtained from commercial retail outlets throughout New Zealand. This provided a total of 291 samples for analysis.

A total of 291 samples tested achieves a 95% confidence of detecting a defect if 1% of samples are defective and randomly taken. The confidence levels for detecting a defect in the individual sample types are:

Seed sprouts: 95 % if there is between 2-3% defective product.
Leafy vegetables: 95% if there is between 2-3% defective product.
Herbs: 95% if there is 5% defective product.

The small number of producers identified required repeat visits to each to obtain the total sample numbers. Each producer was sampled on at least five occasions. Because of the particularly small number of sprout producers, to increase the statistical robustness of the results, sample numbers were increased by the additional 71 samples from commercial

outlets to give a total number of 117 sprout samples. The production source of the 71 extra samples of sprouts is illustrated in Table 3.

A minimum sample size of 120g was required. Samples were placed in sterile plastic bags or if possible left in their original container, chilled and delivered to the appropriate laboratory.

Laboratory Procedure

Each sample was tested for the following pathogens and indicator bacteria:

- *Campylobacter*
- *Escherchia coli*
- *Escherichia coli* O157
- *Listeria monocytogenes*
- *Salmonella*
- Coagulase positive staphylococci

These analytes were selected because:

E.coli provides an indication of faecal contamination and hence the potential risk for contracting a food-borne illness.

Escherichia coli O157, *Salmonella, L monocytogenes* and coagulase positive staphylococci have been found in these types of foods (particularly sprouts) as described in the literature review, and so these organisms were selected as being suitable for analysis.

Campylobacter is a major cause of food borne illness in New Zealand and was included to ascertain the prevalence in hydroponically grown vegetables.

The sprout samples were also examined for *Bacillus cereus* as this organism has been implicated in small outbreaks involving sprouts, in the USA. It was seen as an opportune time to test for the presence of *B. cereus* in sprouts in New Zealand.

The Microbiological Reference Criteria for Food (1995) state in 5.5, Cultured seeds and Grains (bean sprouts, alfalfa etc) and 5.25, Salads-vegetable or fruit-excluding combination with meat, that there should be no *Salmonella* spp or *E coli* present in cultured seeds and/or vegetable salads. The limit for coagulase positive staphylococci in salads is set in 5.25 at 1000/g. These limits were selected for this study. The "General Microbiological Reference Criteria for *Listeria monocytogenes*" does not require raw vegetables to be free of *Listeria* as there is no listeriocidal step in their production. However as outbreaks of listeriosis have been linked to vegetable products it was decided to place a limit of 1000/g of *Listeria monocytogenes* to ensure that any potentially significant number of this organism was recorded. *Bacillus cereus* can be found in vegetable products in low numbers but has also been implicated in outbreaks (National Advisory Committee on Microbial Criteria for Foods,1999). It was decided to place limits of 1000/g for this organism, again to ensure that potentially significant numbers were recorded. *Campylobacter* spp was required to be absent in the samples in this study. These limits are shown in Table 1.

Hydroponic vegetable samples were submitted to ESR Public Health Laboratories at Auckland or Christchurch for analysis. Test methods used were based on those described in the APHA "Compendium of methods for microbiological testing of foods" (1995).

RESULTS

Microbiological Results

Results of microbiological analyses of the 291 hydroponic vegetable samples are summarised in Table 1.

Table 1: Organism Isolation Results (all categories of sample)

Analysis	Limits*	Not Detected	Isolated[#]
Listeria monocytogenes	1000[a]	291	0
Campylobacter	0	291	0
Salmonella	0	291	0
Escherichia coli	0[b]	251	34
Escherichia coli O157	0	291	0
Coagulase positive staphylococci	1000[c]	290	1
Bacillus cereus	1000[c]	117[**]	0

[#] =Isolated (above set limit); [a] = lower limit of detection 100 cfu/g; [b] = lower limit of detection 3 cfu/g; c = lower limit of detection 10 cfu/g; **=only sprout samples were tested for *B. cereus*.

The one coagulase positive staphylococcus detected above the set limit was from a leafy vegetable sample and investigations suggested that this was in fact probably a result of contamination at sampling and therefore may not be significant. There were 3 other isolations of coagulase positive staphylococci from samples that were below the 1000 cfu/g limit, and 3 isolations of *B. cereus* from sprout samples that were below the 1000 cfu/g limit. These isolations were not regarded as significant.

The positive isolations of *E. coli* have been broken down by sample type and these data are summarised in Table 2.

Table 2 *E. coli* isolations by category

Sample	Number tested	Number (%)with *E coli*
Sprout	117	15 (12.8)
Leafy vegetable	114	16 (14.0)
Herbs	60	3 (5.0)
Total	**291**	**34 (11.7)**

The sprout samples were derived from both producers and commercial retail outlets (supermarkets). In Tables 3, 4 and 5 are presented the results of a more detailed *E. coli* isolations from these sprout samples.

Table 3 *E. coli* isolations from sprouts.

Producer No.	Number positive when sampled at producer	Number positive when sampled at supermarket	Total positive
1	0/7	11/49	11/49 (22%)
2	0/5	NS	0/5
3	0/5	NS	0/5
4	0/6	0/2	0/8
5	0/6	0/1	0/7
6	1/6	0/1	1/7 (14%)
7	0/5	NS	0/5
8	2/6	0/2	2/8 (25%)
9	NS	1/1	1/1 (100%)
10	NS	0/4	0/4
Unknown	NS	0/11	0/11
Total	**3/46 (6.5%)**	**12/71 (17%)**	**15/117 (13%)**

NS= Not Sampled

As can be seen in table 4 the majority of E coli isolations (6/15, 40%) from sprouts were in fact from alfalfa sprouts. The remaining 60% were from the other varieties tested, which may also include alfalfa in mixtures or samples not clearly identified as to seed type.

Table 4 *E.coli* isolations from different sprout types

Bean type	No. with *E.coli*	% with *E.coli*
Alfalfa	6/44	17
All other	9/73	12

Table 5 Levels of *E.coli* found in sprouts.

Producer no.	Counts >3 - <20	Counts >20<1100	Counts >1100	Total no. samples tested
1	1 (supermarket)		10 (supermarket)	56
6	1 (supermarket)			7
8	1 (producer)	1 (producer)		8
9			1 (supermarket)	1

HACCP Questionnaire Results

The questionnaire covered all aspects of hydroponic vegetable sprout production from seed purchase through to distribution. The eight sprout producers responses were examined and the control point implementation results are shown in Appendix III.

Analysis of the responses allowed identification of the most common control points and the number of premises not implementing these control points (Table 6).

Table 6 Control Point Non-Implementations

Control Point	Number of Producers Not Implementing
Seed sanitised	6/8
Product washed prior to harvest	6/8
Harvest wash water chilled	6/8
Harvest wash water sanitised	6/8
Chilled distribution	5/8
Seeds certified pathogen free	4/8
Germination water disinfected	4/8
Growing medium sanitised	4/8
Food hygiene training received	4/8
Seeds inspected	3/8
Separation between growing and packing	3/8
Protective clothing worn	3/8

The number of control point non-implementations varied between producers. Table 7 below lists this number per sprout producer.

Table 7 Range of Control Point Non-implementations per Sprout Producer

Number of Control Points Not Implemented	Number of Producers
0-2	0/8
3-4	2/8
5-6	0/8
7-8	2/8
9-10	3/8
11-12	1/8

Microbiological results showed two of the sprout producers had product that contained *E. coli* (Table3). These results have been incorporated into a graph comparing this failure with the number of control points not implemented for each of the producers (Figure1).

Figure 1: Comparison of Microbiological and HACCP Results

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NB The lighter bars (producers 6 & 8) are the two positive *E. coli* producers

DISCUSSION

The microbiological survey of the three vegetable categories produced no isolations of the pathogens tested for. However all three types had *E coli* present (see Table 2) suggesting there is a potential for pathogens from faecal contamination to be present, as *E coli* is a common indicator of such contamination.

For sprouts, the sprouting process creates ideal conditions for the exponential growth of bacteria and if low numbers of pathogens are present on the seed the sprouting conditions may allow for their proliferation. The indicated potential for pathogens to be present in these types of food is a risk to human health that needs to be controlled.

The analysis of the HACCP questionnaire completed by the sprout producers showed there is considerable scope to increase the number of control points used in the hydroponic sprouting and cultivation of these products. The two producers that had *E. coli* present in samples taken directly from their premises had non-implementation rates of control points at the higher end of the scale. This suggests that the more comprehensive the HACCP based management programme is, the lower the risk of a microbiological failure occurring.

All of the outbreaks referred to in Table 8 had contaminated seed as their primary cause. Therefore disinfection of the source seeds is required as a primary CCP. A study (Jaquette *et al*., 1996) has determined that traditional disinfection processes reduced the number of *Salmonella stanley* greatly but did not result in reliable elimination of the organism. If present, *Salmonella stanley* was capable of attaining a population of 10^7 /g during commercial production and handling. These authors investigated both chlorine and heat treatments. A recommended heating step was to expose seeds to a temperature of 57 to 60^{o}C for no more than 5 minutes (or seed viability is lost), and a recommended chlorine wash was at 2,000-4,000 μg/ml. However, even this concentration is not high enough to guarantee removal of this species of *Salmonella*.

Similar results were reported by Beuchat (1997) who found that viable *Salmonella* remained after 10 minutes exposure to 1,800 and 2,000 μg/ml available chlorine from sodium or calcium hypochlorite, 6% hydrogen peroxide or 80% ethanol. While treatment did result in a 1000 fold reduction in numbers it was postulated that bacterial cells are protected from the action of these chemicals due to lodgement in crevices.

The conclusion would appear to be that exposure of seeds to a solution containing approximately 2,000 μg/ml available chlorine is the method of choice. This has been substantiated by investigations of outbreaks in the USA. It was found that the outbreaks were associated with producers not disinfecting seeds, applying treatments inconsistently or using disinfectants at low levels. This can be compared to producers not associated with illness who consistently used seed disinfection with 20,000 ppm calcium hypochlorite. (National Advisory Committee on Microbiological Criteria for Foods, 1999). This aspect of sprout production is under active research.

Of the eight sprout producers surveyed in this study only two sanitise seed before sprouting. It is unclear from the two responses in the questionnaires how frequently the procedure is used or what concentration of chlorine is used. The processes used for the production of sprouted seed offer ample opportunity for cross contamination from a few seeds or sprouts to the entire production lot. (National Advisory Committee on Microbiological Criteria for Foods, 1999).

Recommendation: Seed disinfection should be promoted as the primary defence against contamination of hydroponically cultivated vegetables.

One of the control points identified was the use of seed certified as pathogen free. Although epidemiological investigations frequently identified seeds as the most likely source of contamination of sprouts, laboratory analyses have often been unable to isolate pathogens from implicated seed. This suggests that contamination may be sporadic and at low levels. While a negative result does not guarantee the absence of pathogens, a positive result would allow a producer to avoid using seed lots that have been shown to contain pathogens (National Advisory Committee on Microbiological Criteria for Foods, 1999). Questions must be asked regarding the guarantee of seeds being "pathogen free" in light of these findings and producers should be advised of the limitations of such testing.

The results gathered from this study suggest that leafy vegetables and to a lesser extent herbs also have the potential to contain pathogens. To minimise this risk it is advisable for all producers to implement a HACCP based risk management plan .. Table 12 has a comprehensive list of critical control points that will assist in the safe production of hydroponically grown vegetables.

Recommendation: Hydroponic vegetable growers should adopt HACCP based food safety programmes appropriate for their products.

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APPENDIX I: Literature Review

Hydroponics are defined as "the cultivation of plants, without using soil, by feeding them on chemical solutions". To replace soil inert media (such as expanded clay, growool, Perlite and vermiculite) may be used which contain no plant nutrients in themselves and instead nutrients are supplied in the water used to irrigate the crop. In media-less systems no media are used except for an initial "starter cube".

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In New Zealand a number of hydroponically grown foods are produced but this project focused on lettuce and spinach (collectively termed "leafy vegetables" here), herbs, and seed sprouts. These products are in more intimate contact with the nutrient source than are, for example, tomatoes (although overhead irrigation does occur in some systems). All of these foods may be eaten in an uncooked state and so present a potential vehicle for pathogenic micro-organisms.

Of the hydroponically grown foods seed sprouts have achieved recent prominence with several US regulatory authorities issuing advisories informing people who may be immunocompromised not to eat uncooked sprouts after a recent spate of outbreaks of *Salmonella* and *E. coli* O157:H7 in California. The literature data available are almost entirely related to seed sprouts because of the emergence of outbreaks associated with sprout consumption.

There is some other information available on vegetables in general but this information concerns conventionally grown produce. These areas are therefore covered only briefly as the information may not be wholly applicable to hydroponic vegetables, but it does serve to indicate the types of problems that might occur, primarily due to the use of contaminated water for irrigation or because of contamination during handling. We might expect that the recent rise in awareness of foodborne disease involving fruits and vegetables as the vehicles in the USA will lead to the growth of information in this area. There will also probably be an apparent rise in incidents where fruits and vegetables are implicated because of this increased awareness.

Despite the fact that vegetables are generally regarded as low risk foods it is clear that they can in fact harbour a range of pathogens. Given that these foods are not necessarily cooked before consumption they therefore pose a risk to the consumer.

Seed sprouts have been well researched and hazards are quite well defined. However, no data were found which specifically addressed hydroponic vegetables other than bean sprouts. In addition there appears to be no data for New Zealand produce. This F13 project was timely given the paucity of data available.

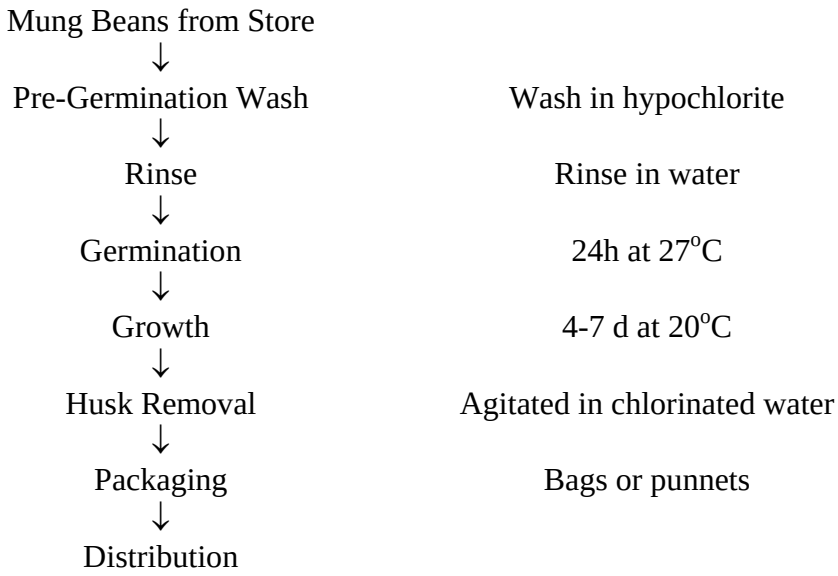
Seed Sprouts

Potential Microbiological Hazards in Production

General Scheme for Seed Sprout Production, e.g. Mung beans

A generalised scheme is shown in Figure 2

Figure 2: General scheme for seed sprout production



Contaminated Seeds

The aerobic plate count (APC) of dry seeds has been reported to vary from $<3.0 \times 10^3$ to 4.0×10^6 /g, and similarly coliforms have been found in high numbers (Propokowich and Blank, 1991; Splittstoesser *et al*. 1983). Since the seeds are soaked before sprouting and seeds contain suitable nutrients to permit microbial growth then any bacteria present on the seeds at this stage will be able to grow and potentially reach high numbers. While Propokowich and Blank (1991) failed to isolate *Salmonella* and *Listeria* from dried seeds, it is evident from the data presented here that a major problem in seed sprouting is the contamination of the original dried seeds. Outbreaks of *Salmonella* and *E. coli* O157:H7 have been attributed to seed contamination and have been detected on unsprouted seeds (e.g. Andrews *et al*., 1979; Itoh *et al*., 1998). However, detection of pathogens on unsprouted seeds is considered difficult (Ponka *et al*., 1995).

Harmon *et al*. (1987) found that, of 98 batches of sprouting seeds (alfalfa, mung bean and buck wheat) intended for domestic use, 57% contained *Bacillus cereus* spores at levels ranging from 3 to >500 /g. However alfalfa and mung bean sprouts were found to be poor substrates for the growth of this organism and so were considered low risk, but buck wheat acted as a good substrate and allowed *B. cereus* to grow to levels where it would be likely to cause food poisoning. These authors also investigated the effect of washing and cooking on

these sprouts and found that washing reduced numbers by around 1 log unit, except with buck wheat sprouts where washing was less effective. Heating sprouts at 95^{o}C for 20 minutes also appeared to be much less effective at lowering the numbers of *B. cereus* on the wheat sprouts when compared to the alfalfa and mung bean sprouts.

Despite these findings a small outbreak due to the presence of *B. cereus* on sprouted soy, mustard and cress seeds has been reported (Portnoy *et al*., 1976) with the organism present at levels as high as 7.6 x 10^7 /g on sprouted mustard seeds. It seems possible that problems with *B. cereus* depend on the species of the seed being sprouted or, possibly, on the strain of *Bacillus* involved.

Sprouting/Germination Process

Salmonella has been shown to grow on mung beans and alfalfa beans during the germination process (Andrews *et al*., 1982), achieving a 3-6 log increase in numbers. *Bacillus cereus* can also grow as described above. Splittstoesser *et al*. (1983) noted a 2 log increase in APC and faecal coliform numbers during the first 2 days of germination/sprouting, but also observed that staphylococci did not grow significantly.

E. coli O157:H7 rapidly increases by 3-5 log cycles during germination (Hara-Kudo *et al*., 1997). Itoh *et al*. (1998) demonstrated that this organism was present on both the outer and inner surfaces of radish sprouts raised from purposely contaminated seeds. Treatment with mercuric chloride of the outer surfaces of the sprouts was insufficient to kill all of the organisms present.

Contaminated Water

Hara-Kudo *et al*. (1997) found that exposure of only the roots to contaminated water resulted in the edible parts of the plant becoming contaminated. Therefore irrigation via the roots only can pose a hazard to the whole of the sprout.

Overall System

One source of information concerning the behaviour of potential pathogens is NASA because of the proposed use of hydroponic systems in long space flights. Such systems are envisaged to act as recycling stations by removing carbon dioxide, producing oxygen and treating “grey water”. It seems that space flight results in a depressed immune system and so renders crew susceptible to pathogens that may be found in their environment (Morales *et al*., 1996). Work has been carried out to determine the ability of pathogens to adhere to the roots of wheat and to grow and proliferate within the rhizosphere both with and without the normal flora. Essentially it was determined that the density and composition of the natural rhizosphere flora played an important role in the ability of potential pathogens to adhere and survive on wheat roots, with most of the organisms tested reducing to levels below the detection limit in the presence of the normal flora.

In other work Garland (1994) found that 70-90% of the bacteria present in a hydroponic system are associated with the rhizosphere. Therefore a circulating water decontamination unit would only kill an average of 20% of the whole system population even if it were 100% efficient. Bacteria were also located in biofilms on equipment surfaces. The author concluded

that control of overall microbial proliferation would be difficult without negatively impacting the health of the plants, and that efforts on control should focus on inhibiting deleterious bacteria in the system. This was not attempted in this study.

Food Poisonings and Reported Microbiological Loading

Outbreaks

Seed sprouts have been identified as the vehicle in numerous outbreaks, some of which are summarised in Table 8 (this is not a comprehensive list of outbreaks). In all cases the source of contamination was determined to have been brought into the growth and harvesting systems on the seeds. For example an international outbreak in the USA and Finland was traced back to seeds supplied by a Dutch company (Mahon *et al*., 1997).

Table 8: **Some Outbreaks of Food Poisoning with Seed Sprouts as the Implicated Vehicle**.

Sprout Type	Pathogen Implicated	Number of Cases	Reference
Mung bean	*Salmonella saint-paul*	143	O' Mahoney *et al*. 1990
Alfalfa	*Salmonella bovismorbificans*	>313	Ponka *et al*. 1995
Alfalfa	*Salmonella stanley*	≥242	Mahon *et al*., 1997
Alfalfa	*Escherichia coli* O157:H7	60	Barrett, E *et al*. 1997
Radish	*Escherichia coli* O157:H7	>6,000	Itoh *et al*., 1998
Soy, mustard and cress	*Bacillus cereus*	4	Portnoy *et al*. 1976

Surveys of Retail Produce

Results of microbiological testing of seed sprouts are shown in Table 9.

The total number of organisms as assessed by the Aerobic Plate Count (APC) is high when compared to other foods, but these figures were not considered to be unusually large for fresh vegetables (Patterson and Woodburn, 1980). Numbers of faecal coliforms appeared to be very high, and a large component of the population were identified as being *Klebsiella*, an organism which is not necessarily derived from faecal contamination and may grow in association with plant material. However the organism is an opportunistic pathogen and may cause disease in people with weakened immune systems.

Potential Hazards in Leafy Vegetable and Herb Production

The range of microbiological hazards associated with these products is primarily associated with the presence of contaminated water or with contamination by a food handler. In conventional production systems the use of uncomposted manure as a fertiliser, or direct contamination from livestock faeces are also sources of contamination Some examples of

outbreaks associated with such products and the sources of the pathogen are given in Table 10.

Table 9: Results of Microbiological Testing of Sprouted Seeds

Sprout (n=sample size)	APC (x 10^8/g)	Total coliforms (/g)	Faecal coliforms (MPN/g)	*Klebsiella* (/g)	Other information	Reference
Alfalfa (n=23)	1.2-13	0.1-620 x 10^5	2.4-240 x 10^5	0.1-36 x 10^6	*Bacillus cereus*, *Yersinia enterocolitica*, *Salmonella* and *Shigella* negative	Patterson and Woodburn, 1980
Bean (n=20)	1.2-13	0.1-250 x 10^5	1.1-240 x 10^5	0.1-72 x 10^6	*Bacillus cereus*, *Yersinia enterocolitica*, *Salmonella* and *Shigella* negative	Patterson and Woodburn, 1980
Alfalfa (n=18)	2.7-22.5	-	3.6->1,100	-	4/18 *Staph aureus* positive	Prokopowich and Blank, 1991
Mixed (n=6)	5.2-30.0	-	3.6->1,100	-	5/18 *Staph aureus* positive	Prokopowich and Blank, 1991
Onion (n=6)	3.6-37.8	-	3.6->1,100	-	4/18 *Staph aureus* positive	Prokopowich and Blank, 1991
Mung (n=5)	0.6-1.1	-	-	-	Fungi isolated	Andrews *et al*. 1982
Alfalfa (n=5)	7.7-8.7	-	-	-	Fungi isolated	Andrews *et al*. 1982
Mung	Mean =1	Mean = 10^7	-	-	0/13 *Salmonella* positive, 2/16 10^4/g *B. cereus*, 14/16 < 10^3/g *B. cereus*	Splittstoesser *et al*. 1983
Alfalfa	0.7-7	1.4-16 x 10^7	-	-	-	Splittstoesser *et al*. 1983
Mixed salad	0.8-9.2	2.5-17 x 10^8	-	-	-	Splittstoesser *et al*. 1983

Table 10: Some Outbreaks of Food Poisoning with Leafy Vegetables and Herbs as the Implicated Vehicle.

Product Implicated	Pathogen Involved	Possible Source(s) of Contamination	Number of Cases	Reference
Lettuce	*Shigella sonnei*	Irrigation with sewage or contaminated drinking water, fertilisation with sewage sludge or compost with human faeces, accidental flooding with contaminated water	110	Kapperud *et al*. 1995
Lettuce	*Giardia*	Contaminated water (?)	42	Grabowski *et al*. 1989
Lettuce	*Escherichia coli* O157:H7	Contaminated fertiliser, runoff from higher fields grazing cattle, cattle contamination of irrigation water, contamination of produce by faeces from other animals (sheep, deer)	40	Anonymous 1998
Lettuce	Hepatitis A virus	-	-	Cited in Tauxe *et al*. (1997)
Green salad	Norwalk-like virus	-	-	Cited in Tauxe *et al*. (1997)
Basil	*Cyclospora*	Food handler	Approx. 300	Pritchett *et al.* (1997)

N.B. These incidents were reported for traditionally grown produce.

Some data for the isolation of pathogens from raw vegetables are presented by Beuchat (1996). Data from this table for vegetables that were the subject of the F13 study are given in Table 11.

It is apparent from the information presented in these tables that vegetables can be significant sources of pathogenic bacteria, protozoa and viruses.

Table 11: Bacterial Pathogens Isolated from Raw Vegetables

Vegetable	Country	Pathogen	Prevalence
Coriander	Mexico	*E. coli* O157:H7	2/10 (20%)
Fennel	Italy	*Salmonella*	4/89 (4.5%)
Leafy vegetables	Malaysia	*Listeria monocytogenes*	5/22 (22.7%)
Lettuce	Italy	*Salmonella*	82/120 (68.3%)
	Lebanon	*Staphylococcus*	14.3%
	Netherlands	*Salmonella*	2/28 (7.1%)
	Spain	*Salmonella*	5/80 (6.3%)
	USA	*Aeromonas*	
Parsley	Egypt	*Shigella*	1/250 (0.4%)
	Lebanon	*Staphylococcus*	7.7%
	Spain	*Salmonella*	1/23 (4.3%)
Salad Greens	Egypt	*Salmonella*	1/250 (0.4%)
	UK	*Staphylococcus*	13/256 (5.1%)
Salad Vegetables	Egypt	*Shigella*	3/250 (1.2%)
	Egypt	*Staphylococcus*	3/26 (8.3%)
	Germany	*L. monocytogenes*	6/263 (2.3%)
	Northern Ireland	*L. monocytogenes*	4/16 (25%)
	UK	*Yersinia enterocolitica*	
Spinach	Spain	*Salmonella*	2/38 (5.2%)
	USA	*Aeromonas*	

Approaches to Safe Hydroponic Vegetable Production

All of the outbreaks referred to in Table 9 had contaminated seed as their primary cause. Therefore disinfection of the source seeds is required as a primary CCP. A study (Jaquette *et al*., 1996) has determined that traditional disinfection processes reduced the number of *Salmonella stanley* greatly but did not result in reliable elimination of the organism. If present, *Salmonella stanley* was capable of attaining a population of 10^7 /g during commercial production and handling. These authors investigated both chlorine and heat treatments. A recommended heating step was to expose seeds to a temperature of 57 to 60^{o}C for no more than 5 minutes (or seed viability is lost), and a recommended chlorine wash was at 2,000-4,000 μg/ml. However, even this concentration is not high enough to guarantee removal of this species of *Salmonella*.

Similar results were reported by Beuchat (1997) who found that viable *Salmonella* remained after 10 minutes exposure to 1,800 and 2,000 μg/ml available chlorine from sodium or calcium hypochlorite, 6% hydrogen peroxide or 80% ethanol. While treatment did result in a 1000 fold reduction in numbers it was postulated that bacterial cells are protected from the action of these chemicals due to lodgement in crevices.

Table 12 Critical Control Point Analysis of Seed Sprout Production

Control Point	Hazard	Control Measure
Raw materials: dried beans	Mould or bacterial growth due to damp storage conditions	Dry storage/ humidity and moisture control of beans
	Contamination by birds, rodents or insects	Pest control programme
	High microbial loading	Use of approved suppliers
	Contamination by foreign matter	Inspection, sieving and washing
	Contamination by pesticides	Discard seeds
packaging materials	Contamination by birds, rodents or insects	Pest control programme
	Contamination by dirt	Clean storage environment
Soaking and Germination of Seeds	Growth of surface microbial contamination	Surface decontamination of seeds
	Contamination from dirty growth containers	Cleaning and disinfection of recycled germination containers
	Contamination from water supply	Disinfection of water supply
Growth of Bean Sprouts	Excessive microbial proliferation	Use of disinfected irrigation water
	Contamination from dirty growth containers	Cleaning and disinfection of recycled germination containers
	Contamination from water supply	Disinfection of water supply
Harvesting/ Washing Sprouts	High microbial levels on the harvested sprouts	Application of control measures before harvesting
	Contamination from wash water	Chlorination of wash water
	Proliferation of micro-organisms in wash tank water	Chilling and chlorination of wash tank water
	Contamination of wash tank surfaces	Cleaning and disinfecting wash tank system daily at the end of production
	Collection bin contamination	Clean/disinfect collection bins
Packing	Contamination from unsanitary handling practices	Personnel hygiene control, regular hand washing, glove changing and cleaning and sanitation of equipment
	Cross-contamination from raw material, germination and growing areas	Well designed factory layout and drainage system. Controlled movement of staff and equipment
	Metal fragments in product	Use metal detector
Storage	Microbial growth	Store at 5^{o}C +/-2. Limited shelf life.
Distribution	Microbial growth	Distribution chill chains at 5^{o}C +/-2.
	Use of out of date product	Date label and stock rotation control
Consumer	Storage abuse of product leading to microbial growth	Clear instructions to the consumer on storage, shelf-life and product preparation.

The conclusion is that exposure of seeds to a solution containing approximately 2,000 μg/ml available chlorine is the method of choice, but it must be borne in mind that this is not a guarantee that all *Salmonella* will be destroyed. This aspect of sprout production is under active research.

Other CCPs (Table 12) were adapted from an internet resource provided by the International Sprout Growers Association (http://www.isga-sprouts.org/haccp.htm). These seem to be quite comprehensive and cover aspects of other vegetables grown using hydroponic systems.

APPENDIX II: HACCP Questionnaire

HYDROPONICALLY GROWN VEGETABLES PROJECT

HACCP QUESTIONNAIRE - SPROUT PRODUCERS

This questionnaire should be completed on the first occasion that the sprout producer is visited.
Please complete a flow diagram of the process at the rear of the questionnaire.

Premises: _____

Operator: _____

Address: _____

Person Interviewed: _____ **Contact Phone Number:**_____

HPO Name: _____ **Date:** _____

Vegetable grown within a protective structure? **Yes** ☎ **No** ☎

SEED

1. From where is seed sourced? _____

2. Is seed certified pathogen free: **Yes** ☎ **No** ☎

   If yes, how is it certified? Specify: _____

   _____

3. Is seed inspected for evidence of contamination on receipt? **Yes** ☎ **No** ☎

4. What type of contamination, if any, is normally observed? Specify: _____

   _____

5. Is seed washed prior to use? **Yes** ☎ **No** ☎

6. Is seed sanitised prior to use? **Yes** ☎ **No** ☎

   If yes, how? _____

_____

SOAKING AND GERMINATION

7. Are containers sanitised between uses? _____ **Yes ☎ No ☎**
8. What type of water supply is used? (*Please circle*)

   Surface/Spring/Well/Bore/Town Reticulated/Other (*Please Specify*) _____

9. Is the water supply disinfected? **Yes ☎ No ☎**

   If Yes, please specify (include normal chlorine level where applicable): _____

_____

GROWING

10. Are animal fertilisers used? **Yes ☎ No ☎**

3. Is the growing medium sanitised? **Yes ☎ No ☎**

   If Yes, please specify method: _____

_____

12. Are growing containers cleaned and sanitised between batches? **Yes ☎ No ☎**

HARVESTING

13. Is product washed prior or post harvest? *(Please tick one box)*

   ☎ Prior ☎ Post ☎ N/A

   If applicable, please outline the process: _____

_____

14. Is wash chilled?

   If Yes, please specify temp: _____

15. Is wash water sanitised? **Yes ☎ No ☎ N/A ☎**

   If Yes, please specify how (include normal chlorine level where applicable): _____

_____

16. Where wash water storage tanks are used, are these sanitised? **Yes ☎ No ☎**

PACKING

17.Are packing materials stored so as to avoid contamination? **Yes ☎ No ☎**

18.Is there separation between growing and packing areas? **Yes ☎ No ☎**

19.Are packing staff appropriately dressed in protective clothing? **Yes ☎ No ☎**

STORAGE

20.Is packed product stored under temperature controlled conditions? **Yes ☎ No ☎**

Please specify temperature: _____

DISTRIBUTION

21.Is product distributed under temperature controlled conditions? **Yes ☎ No ☎**

Please specify temperature: _____

22.Is product date marked? **Yes ☎ No ☎**

23.Is product distributed?

a) Locally **Yes ☎ No ☎**
b) Regionally **Yes ☎ No ☎**
c) Nationally **Yes ☎ No ☎**

STAFF TRAINING

24.Do staff receive food safety training? **Yes ☎ No ☎**

Please specify: _____

BACTERIOLOGICAL TESTING

25.Is bacteriological testing of product or environment carried out? **Yes ☎ No ☎**

If yes, please specify nature and range of test programme: _____

_____

Completed form and flow diagram should be returned to:
Cliff Dawson
Southern Public Health Services
PO Box 1364
Invercargill

APPENDIX III: Hydroponically Grown Vegetables Project

Critical Control Point	Producer Number							
SEED	1	2	3	4	5	6	7	8
1. Grown under cover	Y	Y	Y	Y	Y	Y	Y	Y
2. Certified pathogen free	Y	N	Y	Unknown	N	N	Y	N
3. Inspected	Y	Y	N	Y	Y	N	N	Y
4. Washed	N	Y	Y	Y	Y	Y	Y	Y
5. Sanitised	Y	N	Y	N	N	N	N	N
SOAKING AND GERMINATION								
6. Containers sanitised	Y	Y	Y	Y	Cleaned only	Y	N	Y
7. Water disinfected	Y	N	Y	Y	N	N	Y-Town supply	N
GROWING								
8. Animal fertilisers used	N	N	N	N	N	N	N	N
9. Medium sanitised	Y	N	N	N/A	N	Y	N	Y
10. Containers sanitised between batches	Y	Y	Y	Y	N-Hot water only	Y	Y	Y
HARVESTING								
11. Product washed prior to harvest	N	Y	N	N	N	N	Y	N
12. Product washed post harvest	Y	N	Y	Y	Y	Y	Y	Y
13. Wash water chilled	Y	N	N	Y	N	N	N	N
14. Wash water sanitised	Y	N	Y	N	N	N	N	N
15. Wash water storage tanks sanitised	N	N/A	Y	N/A	N/A	N/A	N/A	N/A
PACKING								
16. Packaging stored safely	Y	Y	Y	N	Y	Y	Y	Y
17. Separation between growing and packing	Y	N	Y	Y	N	N	Y	Y
18. Protective clothing	Y	N	Y	N	Y	N	Y	Y
STORAGE								
19. Chiller storage	Y	Y	Y	Y	Y	N	Y	N
DISTRIBUTION								
20. Chilled distribution	Y	Y	Y	N	N	N	N	N
21. Date marking	Y	Y	Y	N	Y	Y	Y	Y
FOOD HYGIENE TRAINING								
22. Received	Y	N	Y	Y	Y	N	N	N