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# Assessment of irrigation water quality and microbiological safety of leafy greens in different production systems

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## Abstract

Foodborne disease outbreaks associated with fresh produce irrigated with contaminated water are a constant threat to consumer health. In this study, the impact of irrigation water on product safety from different food production systems (commercial to small-scale faming and homestead gardens) was assessed. Hygiene indicators (total coliforms, *Escherichia coli*), and selected foodborne pathogens (*Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7) of water and leafy green vegetables were analysed. Microbiological parameters of all irrigation water (except borehole) exceeded maximum limits set by the Department of Water Affairs, South Africa, for safe irrigation water. Microbial parameters for leafy greens ranged from 2.94 to 4.31 log CFU/g (aerobic plate counts) and 1 to 5.27 log MPN/ 100g (total coliforms and *E. coli*). *Salmonella* and *E. coli* O157:H7 were not detected in all samples tested but *L. monocytogenes* was present in irrigation water (commercial and small-scale farm, and homestead gardens). This study highlights the potential riskiness of using polluted water for crop production in different agricultural settings.

**Keywords**: Foodborne pathogens, Irrigation water, Leafy green vegetables, Microbiological quality, Production systems.

## Practical implications

These results show that the microbial quality of surface water is still deteriorating in different agricultural settings. This may be a possible pre-harvest source of contamination of leafy green vegetables, which may then constitute a health risk to consumers. The presence of *Listeria monocytogenes* along the food supply chain is a potential health risk if the pathogen proliferates leading to high quantities suitable for onset of listeriosis. The absence of *Salmonella* and *Escherichia coli* O157:H7 in the samples tested is an encouraging result. Food safety protocols should be extended to the informal sectors and homestead gardens.

## 1. Introduction

Foodborne disease outbreaks linked with fresh produce irrigated with contaminated water are a persistent health risk to consumers. Herman *et al*. (2015) found that leafy vegetable-associated outbreaks were larger than those attributed to other food types. Contamination of fresh produce can occur either in the pre- or post-harvest environment. Pre-harvest sources include contaminated soil or irrigation water, inadequately composted manure or pesticide sprays reconstituted with contaminated water (Olaimat and Holley 2012). Sources of water used for irrigation can include rain, ground, surface and wastewater (Ijabadeniyi *et al*. 2011; Gelting *et al*. 2015). Presence of pathogens in these water sources has recently attracted attention due to the associated disease outbreaks linking contaminated

water with fresh produce. Irrigation water contaminated with animal feces and human waste is considered one possible route of pre-harvest vegetable contamination (Won *et al*. 2013).

Pathogens including bacteria (Ceuppens *et al*. 2014), viruses and parasites (Tauxe *et al*. 1997) have been isolated from a variety of produce. Among these *Escherichia coli* (enterotoxigenic and enterohaemorrhagic), *Listeria monocytogenes* and *Salmonella* spp. are common contaminants of fresh produce (Burnett and Beuchat 2001). More recently, S*almonella, L*. *monocytogens* and *E*. *coli* (O157:H7) have become the most important species implicated in gastroenteritis outbreaks linked to the consumption of fresh and fresh-cut produce (Olaimat and Holley 2012). Several outbreaks associated with *E*. *coli* O157: H7 in salad containing cabbage and lettuce (Smith De Waal and Bhuiya 2007), *Salmonella* spp. in lettuce (Nygard *et al*. 2008) and other leafy green vegetables (Steele and Odumeru 2004) have been reported.

Leafy green vegetables are an important part of a healthy and nutritious diet and over the past 20 years, their world supply increased by 38% (Kirezieva *et al*. 2015). Leafy greens are also commonly consumed raw as in fresh salads or coleslaw and their safety at the point of sale is therefore important since the final wash or cook step is often not present. Poor sanitation conditions of processing units or kitchens that come into direct contact with the product can further influence microbial quality and product safety. Therefore, water used for irrigation and during processing must be of suitable microbial quality (Pachepsky *et al*. 2011).

Water scarcity (Gemmell and Schmidt 2012), cost and inconsistent supply can lead to the use of non-potable water of uncertain quality for irrigation, which may intensify produce contamination (Gemmell

and Schimdt 2013). During the dry season farmers rely on underground, surface and ground harvested rainwater. In a recent study, Chidamba and Korsten (2015) questioned the microbial safety of ground and rain harvested water. Fecal contaminated irrigation water is often identified as a possible source of pathogens isolated from fresh, ready-to-eat fruit and vegetables (Olaimat and Holley 2012). Traditionally, irrigation water has been extracted from sources with little consideration of quality and safety. Although guidelines exist for quality of irrigation water (DWAF 1996), little attention has been paid to its effective implementation (Gemmell and Schmidt 2013). In this study, microbiological parameters and safety of different food production systems ranging from commercial to small-scale and rural subsistence homestead gardens were compared. The study also assessed the impact of polluted irrigation water and production practices on the safety of the final product as well as the presence of foodborne pathogens in the water used for leafy green vegetables.

## 2. Materials and Methods

### *2.1. Site and sample collection*

The samples were collected from four commercial vegetable farms of different sizes and production intensities, one small-scale subsistence farm, and forty homestead gardens. The sites were selected based on a strategy to assess different production systems in the formal and informal sector all involved in leafy green vegetable production. Different production regions i.e. Gauteng Province (Farm A, B and small-scale), North West Province (Farm C and D) and Eastern Cape Province (homestead gardens) were also targeted. The production systems, water sources and upstream activities have been described in Appendix A Table A.1. In short: All sites produce cabbage except for Farm A that also produces baby spinach and lettuce in an open field large scale commercial production system. Three of the

commercial farms (Farms A, C and D) have onsite pack-houses. Commercial farms A, B and D are Global G.A.P certified according to the Integrated Farm Assurance standard.

Commercial farms (B, C and D) draw water from a nearby river and store the water in a holding tank (Appendix A Table A.1) before irrigation using a center pivot and sprinklers. Borehole water is used for irrigation (center pivot) at farm (A). The borehole water is drawn into a temporary reservoir dam; and then pumped into the irrigation system. The small- scale farmer also uses water drawn from a nearby river and stores it in a container before use. The households use nearby river water which they fetch in buckets to water the cabbages.

Postharvest processing of spinach and lettuce (Farm A) included a chlorine wash (75-80 ppm active chlorine), hand-sorting along a conveyer belt, modified atmosphere packaging (MAP) (4-5% oxygen, 7-10% carbon dioxide) and cold-room storage (4 °C, ≤ 24 h) before transportation to the retailer distribution centers. Cabbages were minimally processed by trimming the outer leaves prior to bulk-bagging (commercial farms), sold without removing leaves (small-scale farm) or directly consumed without removing no more than three outer leaves (homestead gardens)..

Irrigation water ($n$= 142) was sampled from the river, borehole outlets, holding dam and containers, and pack-house wash water using sterile two litre (L) plastic bottles. River and holding dam water samples were collected about 1-2m from the edge avoiding debris. Borehole water samples were collected directly from the outlet pipes. All water samples were collected in three replicates per sampling point. Fresh produce and water samples were collected periodically between 2013 and 2014 to ensure that each site was visited at least twice.

A total of 474 samples comprising of cabbage (*n*= 334), baby spinach (*n*=84) and lettuce (*n*= 56) were collected from commercial, small-scale and homestead gardens. Samples were collected sequentially, following the same crop from the field to the pack-house based on methods as described by Johnston *et al*. (2005), including distribution centres of retailers for produce from Farm A. The homestead product was collected directly at respective homestead gardens. Vegetables were sampled on farm, after transport to pack-house, after preparing for sale and for Farm A, at point of retail distribution. Three cabbage and lettuce leaves were discarded before sampling. The majority of samples collected (70%) consisted of cabbage because this is the preferred and affordable vegetable for the majority of the people in the region (DAFF 2010). All samples were collected aseptically using disposable sterile gloves, placed in sterile brown paper bags, immediately transported to the laboratory in cooler boxes with ice packs and analysed within 24-48 h.

### *2.2. Microbiological and pathogen analyses*

#### *2.2.1 Leafy green vegetables*

Leafy green vegetable samples (25 g), were suspended in 225 ml of 0.1% peptone buffered water (PBW) (Merck, Johannesburg, South Africa) in a blender and macerated for three minutes to release the internal and surface bacteria. Serial dilutions were made in PBW and 100 µl aliquots plated in duplicates onto standard 1nutrient agar (STD1) and Reasoner's 2A (R2A) agar (Merck). The STD1 and R2A plates were incubated at 37 ºC and 25 ºC respectively. Supernatant from macerated fresh produce samples in 0.1% PBW was used to determine presence of total coliforms, *E. coli*, *Enterococci* and *Pseudomonas* with Colilert-18, Enterolert-18 and Pseudolert-18 chromogenic substrate tests kits and Quantitray 2000 trays (Idexx, Westbrook, Maine), as per the manufacturer's instructions. After incubation, the backing material of each Quantitray was disinfected with 70% ethanol. A loop-full of

two fluorescence-positive well contents (per tray) was streaked onto Eosin methylene blue agar (EMBA), *Enterococcus* and *Pseudomonas* selective agar (Merck) (Chidamba and Korsten 2015)

For *Salmonella* and *L. monocytogenes* detection, the macerated produce samples were incubated at 37 °C for 24 h. Afterwards, 1 ml of the pre-enriched broth was inoculated into 9 ml Rappaport-Vasidallis and *Listeria* enrichment broth for selective enrichment of *Salmonella* (Gomba *et al*., 2016) and *L. monocytogenes*, respectively. Rappaport-Vasidallis was incubated at 42 °C for 24 hrs while *Listeria* enrichment broth was incubated at 37 °C for 18-24 hrs. A loopful of each selective enrichment culture with growth was streaked onto Xylose Lysine Deoxycholate (XLD) agar (for *Salmonella* spp.) and Oxford-*Listeria* selective agar (for *L. monocytogenes*) plates. For detection of *E. coli* O157:H7, 1 ml of the pre-enriched broth was streaked on agar chromoID O157:H7 (Merck). All samples were enriched aerobically on an orbital shaker at 150 rates per minute (rpm). Simultaneous pathogen detection was accomplished by enriching macerated vegetable samples in TA10 (Merck) as per methods by Garrido *et al*. (2013). All plates were incubated at 37 °C for 18-24 h.

*2.2.2 Irrigation water*

Irrigation water (750 ml) was concentrated through cellulose nitrate filters (0.45-mm pore size; Sartorius, Gottingen, Germany) and membranes aseptically added to 9 ml of PBW (Merck), and serially diluted. The diluted water samples were subjected to the same microbiological and pathogen detection analysis described above.

All presumptive positive isolates were purified on nutrient agar (Merck) and identities confirmed using matrix-assisted laser desorption ionisation time of flight mass spectrometry (MALDI-TOF-MS) analysis.

### *2.3. Matrix-assisted laser desorption ionisation time of flight mass spectrometry analysis*

Using default settings, the manufacturer's MALDI Biotyper 3.0 software (Bruker Daltonics, Germany) analyzed spectra of the isolates and compares it with reference spectra in the database (Pinto *et al*., 2011). A logarithmic score is generated corresponding to similarity of spectral patterns and interpreted as per manufacturer's instructions. Results are shown as logarithmic (score) quantities (0 to three levels). A logarithmic score ≥2.3 represents a high confidence level species identification, while ≥2.0 and ≥1.7-1.99 denotes species and genus identifications respectively.

### *2.4. DNA Extraction*

Genomic DNA (gDNA) was isolated from pre-enriched culture (TA10, Merck) with a ZM fungal/bacterial DNA miniprep™ kit (Zymo Research Corporation, Inqaba Biotech catalogue # D6005) as per manufacture's specifications, and concentration determined with the Qubit 2.0 Fluorometer (Lifescience Technology, Johannesburg). Extracted gDNA was stored at -20 °C and kept on ice during further experimental procedures.

### *2.5. Multiplex polymerase chain reaction*

*Salmonella*'s invasion protein A (*invA*) gene, *L. monocytogenes*'s transcriptional activator of the virulence factor (*prfA*) gene and the attaching and effacing A (*eaeA*) gene for *E. coli* O157:H7 (Germini, Masola, Carnevali, & Marchelli, 2009) were targeted in this study. The universal 16S rRNA gene was targeted as an internal control of the presence of amplifiable bacterial DNA. The

oligonucleotide primers used in this study were synthesized by Inqaba Biotec (Pty.) Ltd. The sequences and target genes are listed in Appendix A Table A.2. All m-PCR reactions were performed in a Biorad Mycycler™ Thermal cycler. M-PCR kit (Qiagen®) was used for the m-PCR protocol. A volume of 25 µl of each reaction mixture consisted of Qiagen® PCR master mix (containing HotstartTaq® DNA polymerase, m-PCR buffer and dNTP mix); 2 µl of the primer mixture (*invA* primers (Forward (F) and Reverse (R)) 0.5 micro molar (µM), *eaeA* primers (F and R) 0.5 µM, *PrfA* primers (F and R) 0.5 µM and 16S primers (F and R) 0.3µM), 2 µl of sample DNA and nuclease free water. Thermal cycler conditions (Biorad Mycycler™) were as follows: pre incubation at 95 °C for 7 min; 40 cycles consisting of DNA denaturation at 95 °C for 50 s, primer annealing at 54 °C for 40 s, primer extension at 72 °C for 50 s; final elongation at 72 °C for 5 min. PCR products were visualized under a ultra violet (UV) chamber (Viber lourmat, France) following gel electrophoresis on 2 % agarose gel at 85 volts (V) for 1 h using 1X TAE buffer (ethidium bromide staining). Positive and negative controls for the PCR reaction were also included. The positive control contained DNA mixture of three pathogens (*E. coli, L. monocytogenes* and *Salmonella* spp.). The negative control contained nuclease free water.

### *2.6. Statistical analyses*

Statistical analyses, including geometric means, standard deviations, ranges, and medians were conducted with SAS statistical software (version 9.3). Analysis of variance (ANOVA) tests were performed and the least significance difference (LSD) test used to derive statistical differences ($P < 0.05$) of microbial loads between sampling areas.

## 3. Results

### *3.1. Microbiological quality of irrigation water and leafy greens*

Microbial counts of irrigation water and process wash water ($n = 142$) from commercial farms, small-scale and homestead gardens are shown in Appendix A Table A.3. River irrigation water samples from

commercial farms D, B, and C had 9.43, 7.03, and 6.84 log CFU/ml heterotrophic plate counts respectively (Appendix A Table A.3a). River water samples from a small-scale farm used for irrigation had a geometric mean of 7.17 log CFU/ml. Total coliform levels between commercial, small-scale and homestead farming systems were significantly different ($p < .05$). However, the concentration of total coliforms at commercial (Farm B) and small-scale farms were not significantly different. Total coliforms were not detected in borehole and pack-house wash water collected from farm A. In contrast, total coliforms were detected in pack-house wash water collected from commercial farms C (1.23 log MPN/100 ml) and D (2.66 log MPN/100 ml) (Appendix A Table A.3b). Detected *E. coli* levels ranged from 1.85 to 3.87 log MPN/100 ml (Farm D, river and wash water samples) (Appendix A Table A.3c). Geometric mean *E. coli* counts were 1.79 log MPN/100 ml for holding dam water sample from farm A and 2.14 log MPN/100 ml for pack-house wash water from farm D (Appendix A Table A.3c).

A total of 474 produce samples were collected during September 2013 through end of 2014. Leafy green vegetables collected consisted of cabbage (71%), baby spinach (18%). Due to sampling constraints or changes in cultivation schedules, smaller number of lettuce samples was obtained (12%) at the time of sampling. Aerobic plate counts (APC) ranged from a geometric mean of 2.94 to 4.31 log CFU/g (Appendix A Table A.A.4a). Total coliform levels ranged from 0.12 to 4.7 log MPN/100g (Appendix A Table A.4b). Overall geometric mean *E. coli* counts were either not detected or low for market-ready produce items from commercial farms (A and D) (ND to 0.05 log MPN/100g) and highest for small-scale market-ready produce (3.81 log CFU/g) (Appendix A Table A.4c). Total APC levels at farms A and D decreased from the field throughout packing, with geometric means ranging from 3.7 log CFU/g in the field to 3.52 log CFU/g in market-ready samples.

However, there was an increase in APC levels between field and cabbage samples collected in transport crates on its way to the pack-house. Microbial counts of leafy greens between commercial farms, small-scale and homestead gardening were significantly different ($p < .05$). Levels of APC between field and market-ready cabbage from commercial farms (C and D), and small-scale farms either remained constant or significantly increased (Appendix A Table A.4a).

Market-ready cabbage and lettuce tested negative for *E. coli* but was present in baby spinach (0.11 log MPN/100g) samples from farm A (Appendix A Table A.4c). Total coliforms (0.2 log MPN/100g) were detected in produce collected from commercial farms (C and D) and the small-scale farm, as well as the homestead gardens. There was a significant decrease in *E. coli* presence from the field to the pack-house for cabbage samples. Field cabbage *E. coli* (3.87 log MPN/100g) and market ready cabbage (2.84 log MPN/100g) from commercial farm C were higher than farm D, respectively. There was a nonsignificant increase in *E. coli* between cabbage collected from carriage crates from field and processed samples (from 2.4 log to 2.67 log MPN/100g). Generally, *E. coli* on cabbage samples ready for market were lower (0.85 log MPN/100g) than in field samples. Lettuce samples showed a significant decrease in levels of total coliforms from the field to the pack-house (2.7 log to 0.2 log MPN/100g) (Appendix A Table A.4b). However, *E. coli* levels on lettuce samples in the field and carriage crates were not significantly different. Levels of *E. coli* on spinach samples from field to market also decreased significantly (2.35 log to 0.11 log MPN/100g).

### *3.2. Matrix-assisted laser desorption ionisation time of flight mass spectrometry identification of bacterial species*

Selected potential human pathogenic and dominant bacterial species isolated from all water sources ($n = 113$), cabbage ($n = 112$), spinach ($n = 65$), and lettuce ($n = 59$) are shown in Appendix A Table A.5.

In total, 86% of the isolates submitted for identification in MALDI TOF MS were identified. The isolates in the MALDI-TOF MS database are mostly of clinical origin rather than environmental sources. Therefore, environmental isolates absent in the database could not be identified. In total, 60, 40, and 20 *E. coli* strains isolated were from water sources, cabbage and spinach, respectively. The non-Global G.A.P certified commercial and small-scale farms, and homestead gardens accounted for 71% of the total *E. coli* isolates detected. Presence of *L. monocytogenes* in irrigation water (Farm C, small-scale and homestead gardens) and cabbage was confirmed by MALDITOF-MS. Most of the isolates identified (60%) were known common gut, nasal, mouth and skin microflora. Other identified species were plant associated nonpathogenic endophytes (19%), some of which are known biocontrol agents. Environmental isolates that are neither human or plant pathogens accounted for 16% of the isolates (Appendix A Table A.5).

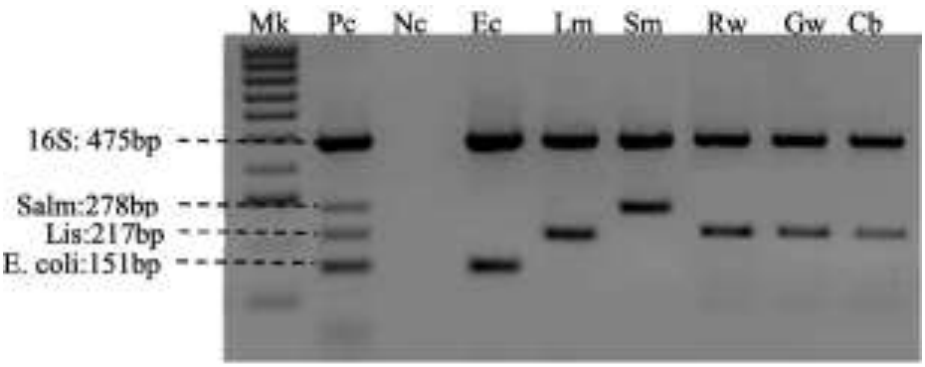


**Figure 1.** Multiplex PCR applied to single and multiple pathogen detection (pc) of *Escherichia coli* O157: H7 (Ec), *Listeria monocytogenes* (Lm) and *Salmonella* spp. (Sm) in river (Rw) and ground harvested water (Gw) and cabbage (Cb) samples. 100 bp DNA ladder (Mk) and negative control (Nc) included

### *3.3. Multiplex PCR*

The Multiplex PCR-based method confirmed the presence of *L. monocytogenes* in river and surface water and in cabbage samples from commercial farm C, small-scale and homestead garden systems

(Fig. 2). At commercial farm C, *L. monocytogenes* was detected in irrigation water, field cabbage and market-ready cabbage samples. *Salmonella* spp. and *E. coli* O157: H7 were not detected in any of the samples tested. Each amplified PCR product generated a unique DNA fragment of the expected size indicating each primer set to be species-specific (Appendix B).

**4. Discussion**

This is the first study where different leafy green vegetable production systems were investigated simultaneously for the presence of waterborne and foodborne pathogens in irrigation water and leafy greens. The study presents the microbial quality of various irrigation water sources (river, borehole, ground harvested rainwater and holding dam) and leafy green vegetables (at commercial and small-scale farms, and homestead gardens). In this study, a multiplex PCR technique was used to simultaneously detect three bacterial pathogens *E. coli*, *L. monocytogenes* and *Salmonella* spp. Multiplex PCR targeting the 16S-23S intergenic region of the *pfrA* gene which encodes a central virulence gene regulator, successfully confirmed *L. monoctytogenes* (Garrido *et al*. 2012).

The microbial quality of river and ground harvested rainwater did not meet the current national standards for irrigation water in South Africa (100 CFU/ml) (DWAF 1996) hence not being suitable for irrigation purposes. Similarly, recent studies have reported poor microbial quality of river (Gemmell and Schmidt 2013) and harvested rain water (Chidamba and Korsten 2015). Conversely, according to the FAO (1989) irrigation water regulations, river and surface water tested in this study would have been considered usable, "fit-for-purpose" and of satisfactory quality to be used during irrigation of leafy greens that are consumed raw. The varied inconsistencies of irrigation water guidelines and regulations render the assessment difficult hence the requirement of a universally implemented minimum standard, suitable for different production systems.

Borehole water was found to meet the current irrigation water standards but holding dam water did not meet this requirement (DWAF 1996). Although *E. coli* (including *E. coli* O157:H7) and total coliforms, *L. monocytogenes* and *Salmonella* spp. were not detected in borehole water at one farm, levels of indicator organisms exceeding current standards were present in the holding dam. Possible sources of contamination could be as a result of dirt, animal droppings and/or run-off water (Pachesky *et al*., 2011; Gemmell and Schmidt 2012; Chidamba and Korsten 2015). The commercial farms visited in this study used overhead irrigation, thereby increasing the probability of direct water-crop contact and potential contamination of produce (Castro-Ibanez *et al.* 2015). *Salmonella* spp. and *E. coli* O157: H7 were not detected in any of the irrigation water sources. Similarly, a study by Holvoet *et al*. (2014) did not detect *Salmonella* spp. and *E. coli* O157:H7 but regarded the irrigation water as a risk factor due to the presence of other human pathogens (*Campylobacter* spp.). In contrast, a recent study reported the presence of *Salmonella* spp. in river water (Gemmell and Schmidt 2013). In our study, *L. monocytogenes* was detected in irrigation water used at one commercial farm (C) and the small-scale farm (river source sample), and homestead gardens (river and ground harvested rainwater), but not at the other farms (A, B and D). Detection of *L. monocytogenes* in irrigation water indicates a potential health risk to consumers if the product gets contaminated and consumed raw, similarly to that described for fresh milk (Van Kessel *et al*. 2011). In South Africa, cabbage is commonly consumed raw in salads such as coleslaw (DAFF 2010).

River water used at farms C and D also had *E. coli* and faecal coliforms levels exceeding current South African standards (DWAF 1996). The commercial farms C and D are located in close proximity to rural areas where sanitary conditions are poor and pit latrines are utilized (observations during sampling) hence river water can be contaminated. This suggestion is similar to a recent report by

Gemmell and Schmidt (2012) that implicated unsanitary conditions and use of pit latrines in water contamination linked with vegetable production systems. Rainfall events increase concentrations of pathogens and indicator organisms in rivers and reservoirs owing to surface run-off (Pachepsky *et al*. 2011). The use of poor microbiological quality irrigation water further increases the risk of contaminating irrigated produce (Aijuka *et al*. 2015) especially when using overhead irrigation (Castro-Ibanez *et al*. 2015). A previous study indicated the potential effect of contaminated irrigation water on pre-harvest vegetables (Ijabadeniyi *et al*. 2011).

Cabbage samples collected from commercial farm C (field, pack-house and market-ready samples) and the small-scale farm (field and market-ready samples) and homestead gardens (on site samples) had *L*. *monocytogenes*. Irrigation water used at these sites was also positive for *L*. *monocytogenes*. These sites were not Global G.A.P certified therefore adherence to proper food safety guidelines is not done. In a recent study by Castro-Ibanez *et al*. (2015), *L*. *monocytogenes* was not detected in a leafy green vegetable (baby spinach) and irrigation water samples but *Salmonella* was present.

Similarly to irrigation water sources, *Salmonella* spp. and *E*. *coli* O157: H7 were not detected in all produce samples tested; a result similar to a study by Koseki *et al*. (2011). The microbial quality of baby spinach, cabbage and lettuce sampled from two Global G.A.P certified commercial farms (A and D) was compliant with current regulatory specifications (DWAF 1996) of fresh produce, despite compromised quality of their holding dam/pond water samples. This is an important point because the use of contaminated irrigation water may not always result in contaminated products (Du Plessis *et al*. 2015). An effective risk based approach is therefore required to appropriately manage any food safety system.

Indicator systems (coliforms and *E. coli*) at sites where foodborne pathogens were not detected were generally lower than recommended standards. Johnston *et al*. (2005) detected low levels of *E. coli* and coliforms on fresh produce. Similar to other findings (Cardamone *et al*. 2015), coliforms and *E. coli* were detected at concentrations complying with current national (DAWF 1996) and WHO (2008) regulations in market ready lettuce and baby spinach, respectively. Contaminated irrigation water is one of the factors influencing microbiological quality of leafy green vegetables (Seow *et al*. 2012). Our study reports *E. coli* prevalence of 18, 20 and 27% for baby spinach, lettuce and cabbage, respectively; this is higher than concentrations previously reported by Cardamone *et al*. (2015). Traditionally, coliforms were used as indicators of faecal contamination and potential presence of pathogenic bacteria (Steele and Odumeru 2004), hence they were tested for in this study. However, the current scientific view is that coliforms alone cannot provide decisive evidence about the occurrence and/or concentrations of most significant pathogens in irrigation water (Pachepsky *et al*. 2015). Similarly, this study cannot establish a correlation between the presence of coliforms and pathogens.

Microbial loads declined significantly from field throughout packing at commercial farms (A and D) with Global G.A.P certification. Furthermore, *Salmonella* spp., *E. coli* and *L. monocytogenes* were not detected in leafy green vegetables, either from the field or from the pack-house on these commercial farms (A and D). Therefore, leafy green vegetables from Global G.A.P certified farms can be considered compliant and in all likelihood safer compared to other farming systems, hence the necessity to improve fresh produce quality by controlling hazards at different stages of production (Uyttendaele *et al*. 2015).

Except for a non-Global G.A.P certified commercial farm C, APC declined from field throughout packing. Lower APC counts are reported in this study, as with previous studies (Johnston *et al*. 2005; Oliveira *et al*. 2010). Overall microbial quality of produce is indicated partly by APC. Although not related to food safety risks, APC acts as an indicator for food quality and that the produce has been exposed to conditions suitable for microbial growth (Oliveira *et al*. 2010). Moreover, the health impact of high APC concentrations is not clear.

Conditions in the pack-house are suitable for proliferation of pathogens (Johnston *et al*. 2005). In a contaminated pack-house, cross-contamination is possible between contact surfaces and produce. Results in this study suggest contamination of faecal origin in the pack-house at farm C where total coliforms and *E. coli* were detected in wash water, along the conveyor belt and on the market-ready samples. Levels of these coliforms and *E. coli* can increase during the processing phase in the pack-house possibly affecting produce shelf-life, as found by previous studies (Castillo *et al*. 2004; Johnston *et al*. 2005). The use of contaminated wash water may also result in further spread of contaminants and cross contamination of the product during wash steps (water dips). Poor sanitary conditions observed at commercial farm C, as well as at the small-scale farm and homestead gardens contribute to the microbiological quality of cabbage samples.

General good agricultural practices recommend treating contaminated irrigation water if not compliant with suitable water fit-for-purpose according to standard requirements (FAO 2008), with farms A and D the only large-scale commercial systems adhering to them. It is important to note that treating water with chlorine reduces populations of pathogenic and other microorganisms but cannot totally eliminate these (Ramesh *et al*. 2003). Although there can be potential risks towards the end of the supply chain

with the detection of total coliforms and *E. coli*, this study cannot establish a direct correlation between *E. coli* as an indicator system and presence of food borne pathogens similar to the findings of Ceuppens *et al*. (2014).

## 5. Conclusion

This study provides baseline information regarding the microbiological quality of irrigation water sources and leafy green vegetables in different agricultural environments. *Salmonella* and *E. coli* O157: H7 were not detected in irrigation water and in any of the leafy greens tested in this study but *L. monocytogenes* were present one commercial and small-scale farm, and homestead gardens. River water and ground harvested rain water sampled in this study exceeded the drinking and irrigation water standards and therefore require treatment prior to use. Sanitary measures in small-scale and homestead production systems require improvement of general food safety measures. Treatment of irrigation water and implementation of good management systems can improve produce quality and safety from field-to-market. This study reiterates that every step from production to consumption affects the microbial quality of produce in all food production systems including commercial, small-scale and rural agriculture. The study also emphasizes that preventing contamination of leafy greens with waterborne pathogens is a significant challenge for food production systems given the poor quality of available water for crop cultivations. In future, the link between water polluted with *L. monocytogenes* and cabbage should be assessed and pathogen genetic diversity investigated.

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# APPENDIX A

**TABLE A.1** Details of sampling sites

| Site | Global G.A.P cert. | Number/ Size (Ha)[a] | Possible upstream pollution source (s) | Irrigation water source (s) | Water storage | Crop (s) | Onsite packhouse |
|---|---|---|---|---|---|---|---|
| Farm A | Yes | 550 | Poultry and | Borehole | Dam | Baby spinach | Yes |
| | | | horse farms | | | Cabbage | |
| | | | | | | Lettuce | |
| Farm B | Yes | 150 | Waste water treatment plant | River | Dam | Cabbage | No |
| Farm C | No | 200 | Informal settlement | River | Tank | Cabbage | Yes |
| Farm D | Yes | 350 | Informal settlement | River | Pond | Cabbage | Yes |
| Small-scale | No | 150 $M^2$ | Waste water treatment plant | River | Tank | Cabbage | No |
| Homestead gardens | No | 1 | Informal settlement | River and | Bucket | Cabbage | No |
| | | | | Ground harvested rainwater | | | |

Abbreviations: Global G.A.P cert., Global G.A.P certification; Ha: Hectare; $M^2$: Square metre;
[a]values are in hectares unless otherwise stated. a 1 Ha represents an average of 25 $m^2$ at 40 households.

**TABLE A.2** Target genes and primers used for selected foodborne pathogen detection using PCR

| Microorganism | Target gene | Primer sequence (5′-3′) | References |
|---|---|---|---|
| *Salmonella* | *invA* | SAL-F: AAT TAT CGC CAC GTT CGG GCA A | Germini et al. (2009) |
| *Enterica* | | SAL-R: TCG CAC CGT CAA AGG AAC C | |
| *Listeria* | *prfA* | LIS-F: TCA TCG ACG GCA ACC TCG G | Germini et al. (2009) |
| *Monocytogenes* | | LIS-R: TGA GCA ACG TAT CCT CCA GAG T | |
| *Escherichia* | *eaeA* | ESC-F: GGC GGA TAA GAC TTC GGC TA | Germini *et al.* (2009) |
| *coli O157: H7* | | ESC-R: CGT TTT GGC ACT ATT TGC CC | |
| Bacterial DNA | 16S | 16S-F: CCT ACG GGA GGC AGC AGT | Germini *et al.* (2009) |
| | rRNA | 16S-R: CGT TTA CGG CGT GGA CTA C | |

**TABLE A.3** Total bacterial loads in various irrigation water sources[a]

**3a. Heterotrophic plate count (CFU/ml)***

| Source | Commercial Farms | | | | S. scale farm | Homestead gardening |
|---|---|---|---|---|---|---|
| | Farm A | Farm B | Farm C | Farm D | | |
| River | – | $7.03 \pm 0.42^{D}$ | $6.84 \pm 0.46^{D}$ | $9.43 \pm 0.99^{B}$ | $7.12 \pm 0.22^{D}$ | $6.27 \pm 0.23^{E}$ |
| Borehole | $3.18 \pm 0.38^{G}$ | – | – | | – | – |
| Ground harvested rainwater | – | – | – | – | – | $7.01 \pm 0.73$ |
| Holding tank | – | – | $5.62 \pm 0.75^{F}$ | — | $7.68 \pm 0.17^{C}$ | $6.27 \pm 1.18^{E}$ |
| Holding dam | $3.3 \pm 0.13^{G}$ | $6.78 \pm 0.92^{DE}$ | – | $10.2 \pm 0.33^{A}$ | – | – |
| Process wash water | ND | – | $2.32 \pm 0.03^{H}$ | $3.23 \pm 0.12^{G}$ | – | – |

**3b. Coliforms***

| Source | Commercial Farms | | | | S. scale farm | Homestead gardening |
|---|---|---|---|---|---|---|
| | Farm A | Farm B | Farm C | Farm D | | |
| River | – | $3.38 \pm 0.02^{A}$ | $3.13 \pm 0.09^{B}$ | $2.72 \pm 0.05^{DE}$ | $3.38 \pm 0.01^{A}$ | $2.88 \pm 0.57^{CD}$ |
| Borehole | $ND^{F}$ | – | – | – | – | – |
| Ground harvested water | – | – | – | – | – | $3.15 \pm 0.21$ |
| Holding tank | – | – | $2.97 \pm 0.06^{BC}$ | – | $3.38 \pm 0.02^{A}$ | $2.88 \pm 0.55^{CD}$ |
| Holding dam | $3.14 \pm 0.27^{B}$ | $3.36 \pm 0.05^{A}$ | – | $2.97 \pm 0.45^{BC}$ | – | – |
| Process wash water | $ND^{F}$ | – | $1.23 \pm 0.01^{G}$ | $2.66 \pm 0.1^{E}$ | – | – |

**3c. *Escherichia coli****

| Source | Commercial Farms | | | | S. scale farm | Homestead gardening |
|---|---|---|---|---|---|---|
| | Farm A | Farm B | Farm C | Farm D | | |
| River | – | $3.38 \pm 0.06^{B}$ | $3.01 \pm 0.06^{C}$ | $2.42 \pm 0.05^{EF}$ | $3.39 \pm 0.02^{B}$ | $2.59 \pm 0.46^{DE}$ |
| Borehole | $ND^{H}$ | – | – | – | – | – |
| Ground harvested water | – | – | – | – | – | $3.21 \pm 0.24$ |
| Holding tank | – | – | $2.73 \pm 0.09^{CD}$ | – | $3.9 \pm 0.24^{A}$ | $2.76 \pm 0.24^{CD}$ |
| Holding dam | $1.79 \pm 0.6^{G}$ | $3.66 \pm 0.16^{BA}$ | – | $2.83 \pm 0.15^{CD}$ | – | – |
| Process wash water | $ND^{H}$ | – | $1.85 \pm 0.03^{G}$ | $2.14 \pm 0.01^{F}$ | – | – |

Abbreviations: -: not done; B. hole: borehole; G. water: ground harvested rainwater; H. dam: holding dam; H. tank: holding tank; ND: Not detected; S. scale: Small-scale; W. water: wash water.

[a]Unless stated otherwise, values are log MPN/100 ml (mean) ± standard deviation ($p < .05$),

*Means with the same letters are not significantly different.

**TABLE A.4** Total bacterial loads in various leafy green produce commodities[a]

**4a. Aerobic plate counts (CFU/100ml)***

| Produce | Sample Point | Commercial farms | | | | S. scale farm | Homestead Gardening |
|---|---|---|---|---|---|---|---|
| | | Farm A | Farm B | Farm C | Farm D | | |
| Cabbage | Field | $3.59 \pm 0.32^{JKL}$ | $3.61 \pm 0.17^{IJK}$ | $3.93 \pm 0.31^{CDEF}$ | $3.70 \pm 0.27^{HIJ}$ | $4.27 \pm 0.1^{A}$ | $3.95 \pm 0.19^{BCDE}$ |
| | Crates | $4.03 \pm 0.03^{BCD}$ | – | $4.26 \pm 0.06^{A}$ | $4.27 \pm 0.08^{A}$ | – | – |
| | Conveyor | $3.9 \pm 0.12^{DEF}$ | – | $4.07 \pm 0.04^{BC}$ | $3.86 \pm 0.12^{GEF}$ | – | – |
| | Market | $3.44 \pm 0.15^{M}$ | – | $4.04 \pm 0.1^{BCD}$ | $3.52 \pm 0.18^{KLM}$ | $4.31 \pm 0.04^{A}$ | – |
| Spinach | Field | $3.86 \pm 0.31^{EFG}$ | – | – | – | – | – |
| | Crates | $4.04 \pm 0.17^{BCD}$ | – | – | – | – | – |
| | Conveyor | $4.09 \pm 0.16^{B}$ | – | – | – | – | – |
| | Market | $3.46 \pm 0.18^{LM}$ | – | – | – | – | – |
| Lettuce | Field | $3.74 \pm 0.25^{GHI}$ | – | – | – | – | – |
| | Crates | $3.79 \pm 0.06^{FGH}$ | – | – | – | – | – |
| | Conveyor | $3.67 \pm 0.14^{HIJK}$ | – | – | – | – | – |
| | Market | $2.94 \pm 0.15^{N}$ | – | – | – | – | – |

**4b. Coliforms***

| Produce | Sample point | Commercial farms | | | | S. scale farm | Homestead gardening |
|---|---|---|---|---|---|---|---|
| | | Farm A | Farm B | Farm C | Farm D | | |
| Cabbage | Field | $2.47 \pm 0.5^{I}$ | $2.60 \pm 0.37^{HI}$ | $5.19 \pm 0.58^{AB}$ | $2.99 \pm 0.02^{F}$ | $4.70 \pm 0.56^{C}$ | $2.66 \pm 0.27^{GHI}$ |
| | Crates | $3.27 \pm 0.41^{E}$ | – | $5.26 \pm 0.06^{A}$ | $5.27 \pm 0.08^{A}$ | – | – |
| | Conveyor | $2.87 \pm 0.1^{FG}$ | – | $4.03 \pm 0.08^{D}$ | $2.86 \pm 0.12^{FG}$ | – | – |
| | Market | $ND^{L}$ | – | $3.24 \pm 0.53^{E}$ | $0.12 \pm 0.31^{L}$ | $4.18 \pm 0.53^{D}$ | – |
| Spinach | Field | $2.70 \pm 0.18^{GH}$ | – | – | – | – | – |
| | Crates | $5.01 \pm 0.17^{B}$ | – | – | – | – | – |
| | Conveyor | $2.09 \pm 0.16^{J}$ | – | – | – | – | – |
| | Market | $ND^{L}$ | – | – | – | – | – |
| Lettuce | Field | $2.70 \pm 1.83^{GH}$ | – | – | – | – | – |
| | Crates | $4.79 \pm 0.06^{C}$ | – | – | – | – | – |
| | Conveyor | $1.67 \pm 0.14^{K}$ | – | – | – | – | – |
| | Market | $0.2 \pm 0.37^{L}$ | – | – | – | – | – |

**4c. Escherichia coli***

| Produce | Sample point | Commercial farms | | | | S. scale farm | Homestead gardening |
|---|---|---|---|---|---|---|---|
| | | Farm A | Farm B | Farm C | Farm D | | |
| Cabbage | Field | $1.83 \pm 0.96^{F}$ | $1.83 \pm 0.78^{F}$ | $3.87 \pm 0.49^{A}$ | $1.98 \pm 0.91^{DEF}$ | $3.95 \pm 0.76^{A}$ | $2.27 \pm 0.55^{CDE}$ |
| | Crates | $2.08 \pm 0.41^{DEF}$ | – | $4.06 \pm 0.06^{A}$ | $1.87 \pm 0.08^{EF}$ | – | – |
| | Conveyor | $0.87 \pm 0.01^{GH}$ | – | $3.03 \pm 0.08^{B}$ | $1.13 \pm 0.08^{G}$ | – | – |
| | Market | $ND^{I}$ | – | $2.84 \pm 0.1^{B}$ | $0.05 \pm 0.14^{I}$ | $3.81 \pm 0.04^{A}$ | – |
| Spinach | Field | $2.35 \pm 0.69^{CD}$ | – | – | – | – | – |
| | Crates | $2.62 \pm 0.17^{BC}$ | – | – | – | – | – |
| | Conveyor | $1.09 \pm 0.16^{GH}$ | – | – | – | – | – |
| | Market | $0.11 \pm 0.31^{I}$ | – | – | – | – | – |
| Lettuce | Field | $2.33 \pm 0.7^{CD}$ | – | – | – | – | – |
| | Crates | $2.39 \pm 0.06^{CD}$ | – | – | – | – | – |
| | Conveyor | $0.67 \pm 0.14^{H}$ | – | – | – | – | – |
| | Market | $ND^{I}$ | – | – | – | – | – |

Abbreviations: -, not done; ND, not detected; S. scale, small scale.
[a]Unless stated otherwise, values are log MPN/100 g (mean) ($p < .05$),
*Means with the same letters are not significantly different.