PHYLLO-EPIPHYTIC AND ENDOPHYTIC PATHOGENS ON *BRASSICA OLERACEA* VAR. *CAPITATA* L. AND *SPINACIA OLERACEA* L. AS AFFECTED BY SMALL-SCALE FARM PRODUCTION SYSTEMS

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Abstract. The phyllosphere hosts a considerable number of microorganisms, providing a vast habitat for naturally associated phyllobacteria due to its topography as it offers various colonization and infiltration sites. Contamination of vegetables may occur through pre-harvest and post-harvest activities and include cross-contamination from infected personnel. The study aimed to examine the prevalence of microbial contamination for spinach (*Spinacia oleracea* L.) and cabbage (*Brassica oleracea* var. *capitata* L.) at various farms based on the production and agronomic systems in South Africa, Free State. In addition, the study further demonstrated that several potentially pathogenic microorganisms are present in common fresh leafy greens such as spinach and cabbage. Almost all the analyzed and identified microorganisms were reported to be opportunistic pathogens. Spinach and cabbage phyllosphere were contaminated mostly with *Staphylococcaceae, Pseudomonadaceae, Morganellaceae, Caulobacteraceae, Moraxellaceae, Burkholderiaceae, Yersiniaceae, Xanthomonadaceae, Listeriaceae* and total coliform species. The predominant genera were *Staphylococcaceae, Morganellaceae* and *Pseudomonadaceae* in spinach and cabbage isolates. The analysed isolates revealed a high level of contamination by opportunistic pathogens such as total coliform, *Morganellaceae* and *Staphylococcaceae* reflecting a deficit of good agricultural production systems and hygiene practice. From the study, the authors could also demonstrate the rapid rate at which these pathogens can spread through the food chain and cause food poisoning. It is concluded that the bacterial contamination frequency and degree in this present study was significantly considerable and recommended that these vegetables be thoroughly washed before any consumption especially when consumed uncooked, specifically in green salad. Moreover, preparing cooked meals using these leafy vegetables would be better.
Keywords: *spinach, cabbage, consumption, contamination, farm activities, foodborne illness, food safety*

Introduction

The phyllosphere is a natural environment, a leaf surface which is nutrient-rich and consists of complex and diverse microorganisms, bacteria being the most numerous colonists and population (Bashir et al., 2022). Epiphytes are microorganisms that are easily removed from their environment by either disinfectant wash treatment, and endophytes are those that remain and internalize (Micci et al., 2022). Microorganisms are common residents of the phyllosphere and are termed native microflora (epiphytes) and endophytic bacteria (Dees et al., 2015). However, their interaction with other

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microorganisms can be pathogenic, synergistic or antagonistic. Pathogenic or non-pathogenic bacteria have several opportunists strategies they utilize as to contaminate fresh vegetables, at the field or at the time of consumption (Nithya and Babu, 2017). Environment microbe-microbe interactions with the crop microbe including their mechanisms are pivotal for the establishment of opportunistic pathogenic bacteria on the crop (Kiselev, 2022). Various strategies include contamination capability, pathogen interaction, microbial dominance, biofilm and transition including endophytic survival utilizing various defense mechanisms.

Biofilms may represent about 80% of the total microbial population on the phylloplane (Moitinho et al., 2020). The sources that contribute to the habitation of microorganisms in the phyllosphere are the atmosphere, insect, seed, or animal-borne sources including nutrients (Whipps, 2008). The bacterial interactions on the phyllosphere can have a significant effect on the fitness of plants by either promoting plant growth and suppressing or stimulating the colonization of plant and human pathogens (Moitinho et al., 2020).

Minimal processing generally includes peeling, hand preparation, size reduction, defect, sorting using different objects, washing and packaging. Harvesting of produce may be through mechanical harvesters in large operational plants and by hands in small-scale operational plants from the receiving point to the packaging and distribution. Minimal processing is known to be the onset of a produce physiological change due to cutting and trimming exposing conducive sites that provides nutrients to toxic microorganisms.

Food poisoning occurs when contaminated food is consumed, the food may be infected by toxins from toxic microorganisms such as *staphylococcus aureus*, *E. coli* or L*isteria* species (Hernández-Cortez et al., 2017). In addition to that, contamination may be through anthropogenic activities such as poor sanitation and poor hygiene practices including improperly treated irrigation water or poor agricultural practices either as a result of personnel or agronomic devises utilized during minimal processing. Toxins are toxic elements within a bacteria that inflict pathogenic traits depending on the type of toxin, when ingested through contaminated food they manipulate the human immune system resulting in gastrointestinal infection and other severe complications (Ghazaei, 2022). Bacteria utilizes virulence mechanisms such as toxin production to cause a microbial infection or disease condition. Toxins and virulent factors are responsible for the pathogenesis of opportunistic pathogens, they cause human infection which are characterized by severe complications and symptoms including vomiting, diarrheal, abdominal cramp leading to an illness (Abebe et al., 2020). The transfer of pathogens from one composition to the other is always the cause of cross-contamination. If conducive conditions exist some of these pathogens can grow, colonize and form a biofilm. If the food is ingested, the toxin is released, and possible food poisoning can occur. Risk assessment in minimal processing is crucial since biohazards pose a threat to human health.

Fresh vegetable phyllosphere normally carries natural non-pathogenic epiphytic microorganisms (Mulaosmanovic, 2021; Motshabi et al., 2021). In addition to that, microbial communities on the phyllosphere differ in species, composition, dominance and nutrition required. A particular study utilized 16S rRNA gene-directed PCR-DGGE to compare the phyllosphere communities of seven different plant species and the major finding was that microbial phyllosphere communities were more complex than previously thought (Laforest-Lapointe and Whitaker, 2019).

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Microbes that flourish and thrive on the phyllosphere interact with the host which in turn shapes the niche allowing growth of population (Dees et al., 2015). Phyllosphere bacteria may include those bacteria that are pathogenic to the plant (Moitinho et al., 2020). Antagonist bacteria work against pathogens by preventing their growth while biological control agents or biocontrol agents helps in promoting plant health and reduces the severity of a disease termed (Beattie, 2006). Native microflora are naturally present in the phyllosphere and are assumed to play an important role against phytopathogens by activating a defense mechanism (Iqbal et al., 2023). This mechanism is based on their activity of being the potential antagonist agent against enteropathogens. Plant health depends on these phyllosphere bacteria as they a potential effect against human pathogenic microorganisms which are a major threat to food production including ecosystem stability (Adomako and Yu, 2023).

Gram-negative microbiota dominates the phyllosphere with *Pseudomonas* spp. being dominant in a range of 50-80% of the microbial population, this increases the chance of pathogens persisting on the phyllosphere (Sohrabi et al., 2023). Between 30% and 50% of the human population carries *Staphylococcus aureus* as commensal bacteria, contamination from this pathogen can occur through improper handling (Tigabu and Getaneh, 2021; Le Loirs et al., 2003). Furthermore, laboratory experiments with various cultures have revealed many active mechanisms by which bacteria can impair or kill other microbes. Pathogens can occasionally be outcompeted by native bacteria, but the adaptation and interaction depend on specific needs between the plant and bacteria. Pathogenic microorganisms such as bacteria and viruses are the most common cause of food poisoning (Australian Institute of Food Safety, 2021). The battle against bacterial foodborne diseases is facing new challenges because of rapidly changing patterns of human consumption, the globalization of the food market including climate change (Argaw and Addis, 2015).

Cabbage and spinach are highly susceptible to microbial contamination and farm operations comprise several units which are likely to provide opportunities for potential cross-contamination. In light of this, leafy green vegetables are not subjected to any lethal process which is mostly employed to effectively kill pathogenic organisms. It is hypothesized that pre-harvest and post-harvest factors contribute to the amplification of pathogenic microorganisms. The absence of appropriate transportation, good agronomic practices and good hygiene practices including adequate storage and cooling compromises markets quality and food safety. The objective of the study is enumerate microbiota and identify microbial species isolated from spinach and cabbage at small-scale farm level by analyzing spinach and cabbage and storing crates before distribution to various destinations.

Material and methods

Study area and sampling technique

Sample collection

The present study was conducted by procuring sixty samples of raw unpackaged spinach phyllophere and seventy-five samples of cabbage head from different four and five farms, respectively in different local municipality districts within the Free State province, South Africa. The selected farms represent the major small-scale farms which supplies most leafy greens to various buyers making the results of the study representative. Spinach and cabbage were chosen due to their minimal processing

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production, demand and purchase. The farms selected are small-scale farms that supply small villages, black markets such as street vendors, informal markets, guest houses and local supermarkets, and farm-to-farm exchange which is termed intra-farm exchange including some privately owned retails and other neighboring districts.

Samples was collected in the following towns in the Free State Province, South Africa: Motheo District - Mangaung Metropolitan (Farm 1 - 29.1217°S, 26.2128°E), Lejweleputwa District - Matjhabeng Local Municipality (Farm 2 - 28.9784°S, 27.0264°E), Thabo Mofutsanyana District – Setsoto Municipality (Farm 3 - 28.9093°S, 27.5555° E [Spinach samples were not available during sampling season for farm 3]), Fezile Dabi District - Moqhaka Local Municipality (Farm 4 - 27.6373°S, 27.2323°E) and Thabo Mofutsanyana District – Dihlabeng Local Municipality (Farm 5 - 28.2423°S, 28.3111°E). All farms were selected based on the centralized market in Bloemfontein. The market requirement is based on the Good Agricultural Practices (GAP), and at most, the selected farmers followed a similar production system to meet the markets specifications (Mahlangu et al., 2020). A random sampling study design was conducted on spinach and cabbage samples from three different sections, the middle part and two sides of the stored samples ready for purchase. To ensure sample collection was random and representative, at least five areas were assessed for sampling. The samples collected were selected based on the random sampling method, part of the sampling technique in which each sample has an equal probability of being chosen. A sample chosen randomly is meant to be an unbiased representation of the total population.

Fresh leafy spinach and cabbage samples were analyzed for each of the following microorganisms or microbial species: total aerobic mesophilic bacteria, total coliforms, coagulase-positive *Staphylococci* and *Listeria*. All samples were collected aseptically and subsequently transported to the laboratory and were prepared, plated on various presolidified agars from the homogenate of the samples prepared and incubated within 12 h, the same day of collection.

Microbiological analysis

Sample preparation

Cabbage samples were cut into quarters, and one-quarter of each batch was taken for processing and bacterial identification. The quarters were coarsely chopped and combined in a sterile hood to avoid contamination. Two opposing segments were taken and the other two were discarded. The remaining segments are mixed and further reduced in the same way representative of the whole (Annor, 2009; Moloantoa et al., 2023). The ready-to-be-purchased spinach phyllosphere were washed, chopped and roughly mixed and 25 g portions of both samples were weighed and shaken in 90 ml of sterile buffered peptone water (BPW) for 3 min before samples were homogenized (Annor, 2009).

In this present study, a total of 25 g of each collected samples were added to 90 ml of sterile peptone water solution (Merck, Republic of South Africa) and homogenized in a stomacher (Stomacher® 400 circulation Seward, Lasec, Republic of South Africa) for 260 rpm for 1 min. Then, the mashed samples were filtered through a sterile folded paper filter (Lasec, Republic of South Africa). The sequential dilutions were prepared using filtrated samples for plate count analyses. Subsequently, serial dilutions of up to 10^5 folds of the homogenate were prepared for each sample and utilized for bacterial analysis. Serial dilutions of the samples were made in 0.1% buffered peptone water; 0.1 ml from each dilution (10^{-1} to 10^{-5}) was pipetted and spread plated in duplicates on

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a standard pre-solidified agar medium and incubated at 32°C for 72 h. After incubation, plates with colonies from 30 to 300 were counted.

Aerobic mesophilic count, *Enterobacteriaceae* count (total coliform), *Staphylococci* count, and *Listeria* count were enumerated from the homogenate of the samples prepared. Plate count agar including selective media such as MacConkey with salt, MacConkey without salt, and Baird-Parker supplemented with egg yolk (Merck, Republic of South Africa) and Brilliance chromogenic *Listeria* (ThermoFisher, Scientific, Republic of South Africa) were selected. The isolated colonies were counted using an 80 Scan 1200® Automated Colony Counter (Interscience). The mean number of colonies counted for all count types was expressed in log colony forming units (CFUs). Isolates were further characterized biochemically using API 20E for *Enterobacteriaceae* and related genera whilst API 20NE was utilized for the identification of non-fastidious and non-enteric Gram-negative rods. API STAPH was utilized for *Staphylococci*, *micrococci* and related genera and API *Listeria* for identification of *Listeria*. The tests were performed according to the manufacturer's instruction (Biomerieux, Republic of South Africa).

Total aerobic mesophilic count

The enumeration of the total viable aerobic mesophilic count was determined by plate count using the standard plate count agar (PCA) medium. Samples were serially diluted in buffered peptone water (BPW) and then, aliquots of 0.1 ml were inoculated in plate count spread-plate technique, following incubation at 37°C/48 h (Shalini, 2010).

Enterobacteriaceae count (total coliform)

To count the members of *Enterobacteriaceae*, 0.1 ml of 10^1–10^5 serial dilution of the leafy green vegetable samples was spread plated on MacConkey agar containing salt and MacConkey agar without salt. Plates were incubated at 32°C for 24 h after spreading. Colonies were counted as members of *Enterobacteriaceae* (Spencer and Spencer, 2001).

Staphylococcus spp. count

The enumeration of coagulase-positive *Staphylococci* was performed using Baird-parker agar (BPA) plus egg yolk and potassium tellurite following serial dilution in BPW. BPA plates will be incubated at 37°C/48 h and checked for typical/atypical colonies (black, shiny, convex, and surrounded or not by clear zones, 2-5 mm). Between 5 and 10 typical and atypical colonies were purified in blood agar plates. Results were expressed based on the number of coagulase-positive *Staphylococci* on plates (Acco et al., 2003).

Colonies were streaked out on plate count agar plates and blood agar for pure colonies before being analyzed using API 20E, API 20NE and API STAPH for identification of organisms (Biomerieux, Republic of South Africa). Briefly, 1-4 colonies of identical morphology from young cultures (18-24 h) were picked and emulsified in 5 mL of sterile sodium chloride (0.85%) for API 20E, API STAPH and 20NE and the turbidity adjusted to the equivalent of the turbidity of 0.5 McFarland standards. The standardized bacterial suspension was distributed into the tubes of the test strip carefully to avoid the formation of bubbles. Anaerobiosis was created by overlaying with sterile mineral oil and the strips were subsequently incubated in a humid atmosphere for 18–24 h at 37°C.

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An additional oxidase test was performed for *Pseudomonadaceae* by adding 2-3 drops of reagent directly to suspect colonies on the nutrient agar plate. The color change was observed within 10 s. When using Kovac's Oxidase reagent, microorganisms are oxidase-positive when the color changes to dark purple within 5 to 10 s. Microorganisms are delayed oxidase positive when the color changes to purple within 60 to 90 s. Microorganisms are oxidase-negative if the color does not change, or it takes longer than 2 min.

Listeria count

For the isolation of *Listeria* spp. approximately 25 g of each sample was homogenized with *Listeria* broth and stomach for a minimum of 30 s to mix the sample. Incubate the broth without agitation at 30°C for 24 ± 2 h. Gently agitate the bag then, using a microbiological loop, remove 0.1 ml and inoculate onto *Brilliance Listeria* Agar plate (chromogenic). Carefully spread the inoculum as soon as possible over the surface of the plate using a sterile spreader without touching the sides of the plate with the spread. Invert the inoculated plates so that the bottom is uppermost and incubate at 37°C for 24 ± 2 h. Examine the plate for blue colonies with and without opaque white halos (ISO 16140 standard). Observe the type of hemolysis and record it, this qualifies as an additional test. Colonies were streaked on blood agar for pure colonies before confirmed through biochemical identification using an API *Listeria* system (Biomerieux, Republic of South Africa).

For *Listeria* after suspension with a turbidity of 1 McFarland, hemolysis was observed and recorded on the result sheet. After the distribution of suspension into the tube followed by incubation reagents were added and results were recorded again. Add a drop of ZYM B reagent to the test. Data interpretation was performed using the API database with the apiwebTM identification software to obtain the identification result for each strain tested.

Data analysis

Statistical analysis of the data on growth medium influencing the prevalence of pathogens and microbial count mean was performed using the general linear model of SAS software 9.2 version to determine the analysis of variance (ANOVA). Turkey's least significant difference (LSD_T) described by Steel and Tourie (1980) was utilized to determine the significant results between variants. Statistical difference between treatment means was determined at the ($p \leq 0.05$) probability level. The Shapiro-Wilks test was performed on standardized residuals to test for any deviations from normality (Shapiro and Wilk, 1965). The growth medium influencing the prevalence of pathogens and microbial count mean were subjected to multivariate data analysis, using principal component analysis (PCA-XLSTAT 2015) to identify and evaluate the groupings between the variables.

Results and discussion

Cabbage phyllosphere microbial count concentrations

Analysis of variance (ANOVA)

Significant interactions between the farms and concentrations were observed in all five concentrations of microbial mean count in different farms (*Tables 1* and *2*).

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Table 1. *Mean log_{10} cfu/ml of bacteria sampled from cabbage phyllosphere from different farms*

Concentrations (log_{10} cfu/ml)			
Farms	**10^{-1} Cons**	**10^{-2} Cons**	**10^{-3} Cons**
Farm 1	$61.25 \pm 26.09^{c,d}$	45.00 ± 21.86^{d}	26.50 ± 14.70^{c}
Farm 2	84.62 ± 30.45^{b}	65.75 ± 26.03^{b}	$44.12 \pm 20.27^{a,b}$
Farm 3	47.25 ± 17.24^{d}	39.12 ± 10.13^{d}	29.50 ± 6.84^{c}
Farm 4	99.37 ± 13.31^{a}	80.00 ± 15.80^{a}	48.75 ± 22.24^{a}
Farm 5	$70.87 \pm 24.23^{b,c}$	59.25 ± 25.40^{c}	$34.00 \pm 15.56^{b,c}$
LSD_T	*14.12	*6.18	*14.57
F-Value	48.01	65.71	28.29
P-Value	< .0001	< .0001	< .0001
Agar			
BP	52.70 ± 20.75^{c}	39.80 ± 15.20^{c}	23.60 ± 7.54^{b}
PCA	95.80 ± 16.26^{a}	79.80 ± 15.02^{a}	57.30 ± 18.82^{a}
WOS	69.00 ± 30.11^{b}	54.70 ± 26.85^{b}	31.30 ± 13.81^{b}
WS	73.20 ± 29.60^{b}	57.00 ± 23.22^{b}	34.10 ± 10.79^{b}
LSD_T	*12.63	*5.53	*13.03
F-Value	46.35	83.69	81.98
P-Value	< .0001	< .0001	< .0001
Farms x Agar			
Farm 1 x BP	28.00 ± 5.56^{i}	20.50 ± 2.12^{g}	12.50 ± 0.70^{e}
Farm 1 x PCA	$96.00 \pm 2.82^{a,b,c,b}$	$75.50 \pm 4.94^{c,d}$	$48.50 \pm 9.19^{b,c,d}$
Farm 1 x WOS	$63.50 \pm 7.77^{e,f,g}$	49.00 ± 2.82^{e}	$22.50 \pm 2.12^{c,d,e}$
Farm 1 x WS	$57.50 \pm 0.70^{e,f,g,h}$	35.00 ± 4.24^{f}	$22.50 \pm 0.70^{c,d,e}$
Farm 2 x BP	$43.00 \pm 5.56^{f,g,h,i}$	33.00 ± 1.41^{f}	$20.50 \pm 2.12^{d,e}$
Farm 2 x PCA	113.00 ± 11.31^{a}	$89.50 \pm 9.19^{a,b}$	$70.50 \pm 9.19^{a,b}$
Farm 2 x WOS	$106.50 \pm 12.02^{a,b}$	$87.50 \pm 6.36^{a,b,c}$	$50.00 \pm 7.07^{b,c}$
Farm 2 x WS	$76.00 \pm 2.82^{c,d,e}$	53.00 ± 8.48^{e}	$35.50 \pm 3.53^{c,d,e}$
Farm 3 x BP	$44.50 \pm 6.36^{f,g,h,i}$	34.00 ± 2.82^{f}	$28.00 \pm 1.41^{c,d,e}$
Farm 3 x PCA	$68.50 \pm 2.12^{d,e,f}$	55.00 ± 5.65^{e}	$38.00 \pm 9.89^{c,d,e}$
Farm 3 x WOS	$41.00 \pm 4.24^{f,g,h,i}$	33.50 ± 2.12^{f}	$28.50 \pm 0.70^{c,d,e}$
Farm 3 x WS	$35.00 \pm 26.87^{h,i}$	34.00 ± 1.41^{f}	$23.50 \pm 2.12^{b,c,d,e}$
Farm 4 x BP	$83.00 \pm 8.48^{b,c,d,e}$	57.50 ± 10.60^{e}	$31.00 \pm 8.48^{c,d,e}$
Farm 4 x PCA	$104.00 \pm 4.24^{a,b,c}$	93.00 ± 2.82^{a}	82.00 ± 14.14^{a}
Farm 4 WOS	$96.00 \pm 0.70^{a,b,c,d}$	$80.00 \pm .48^{b,c,d}$	$40.50 \pm 9.19^{c,d,e}$
Farm 4 x WS	114.00 ± 11.31^{a}	$89.50 \pm 4.94^{a,b}$	$41.50 \pm 4.94^{b,c,d}$
Farm 5 x BP	$65.00 \pm 4.24^{e,f,g}$	54.00 ± 4.24^{e}	$26.00 \pm 2.82^{c,d,e}$
Farm 5 x PCA	$97.50 \pm 0.70^{a,b,c}$	$86.00 \pm 1.41^{a,b,c}$	$47.50 \pm 7.77^{b,c,d}$
Farm 5 x WOS	$37.50 \pm 0.70^{g,h,i}$	$23.50 \pm 4.94^{f,g}$	15.00 ± 2.82^{e}
Farm 5 x WS	$83.50 \pm 7.77^{b,c,d,e}$	73.50 ± 4.94^{d}	$47.50 \pm 6.36^{b,c,d}$
LSD_T	*28.25	*12.37	*29.14
F-Value	9.45	16.13	8.81
P-Value	< .0001	< .0001	< .0001

Means followed by the same letter in the same column are statistically non-significant ($P < 0.05$); ns = not significant; * = significant.
Abbreviations: WS = MacConkey with salt; WOS = MacConkey without salt; PCA = Plate count agar; BP = Baird parker

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Table 2. *Mean log_{10} cfu/ml of bacteria sampled from cabbage phyllosphere from different farms*

Concentrations (log_{10} cfu/ml)		
Farms	**10^{-4} Cons**	**10^{-5} Cons**
Farm 1	13.50 ± 13.66^{b}	9.00 ± 2.82^{c}
Farm 2	28.50 ± 19.27^{a}	14.37 ± 10.92^{b}
Farm 3	11.00 ± 4.81^{b}	9.00 ± 2.82^{c}
Farm 4	$21.85 \pm 19.37^{a,b}$	33.50 ± 3.53^{a}
Farm 5	12.75 ± 9.26^{b}	14.00 ± 4.08^{b}
LSD_T	*11.14	*4.71
F-Value	14.53	45.43
P-Value	< .0001	0.0014
Agar		
BP	12.12 ± 3.83^{a}	6.50 ± 3.53^{c}
PCA	32.80 ± 19.77^{b}	18.60 ± 11.93^{a}
WOS	12.20 ± 12.04^{b}	14.00 ± 1.41^{b}
WS	12.70 ± 8.49^{b}	11.75 ± 6.70^{b}
LSD_T	*9.95	*4.47
F-Value	35.75	25.71
P-Value	< .0001	0.0045
Farms x Agar		
Farm 1 x BP	ND	ND
Farm 1 x PCA	$29.50 \pm 12.02^{b,c}$	$9.00 \pm 2.82^{c,d}$
Farm 1 x WOS	3.50 ± 0.70^{e}	ND
Farm 1 x WS	$7.50 \pm 2.12^{d,e}$	ND
Farm 2 x BP	$13.50 \pm 2.12^{b,c,d,e}$	6.50 ± 3.53^{d}
Farm 2 x PCA	54.50 ± 54.50^{a}	31.00 ± 1.41^{a}
Farm 2 x WOS	$33.50 \pm 2.12^{a,b}$	$14.00 \pm 1.41^{b,c}$
Farm 2 x WS	$12.50 \pm 12.02^{b,c,d,e}$	6.00 ± 1.41^{d}
Farm 3 x BP	$12.00 \pm 5.65^{b,c,d,e}$	ND
Farm 3 x PCA	$14.50 \pm 3.53^{b,c,d,e}$	$9.00 \pm 2.82^{c,d}$
Farm 3 x WOS	$11.00 \pm 7.07^{c,d,e}$	ND
Farm 3 x WS	$6.50 \pm 0.70^{d,e}$	ND
Farm 4 x BP	$14.50 \pm 3.53^{b,c,d,e}$	ND
Farm 4 x PCA	52.00 ± 12.72^{a}	33.50 ± 3.53^{a}
Farm 4 x WOS	$10.00 \pm 2.82^{c,d,e}$	ND
Farm 4 x WS	$11.00 \pm 1.41^{c,d,e}$	ND
Farm 5 x BP	$8.50 \pm 3.53^{c,d,e}$	ND
Farm 5 x PCA	$13.50 \pm 0.70^{b,c,d,e}$	$10.50 \pm 0.70^{c,d}$
Farm 5 x WOS	3.00 ± 1.41^{e}	ND
Farm 5 x WS	$26.00 \pm 2.82^{b,c,d}$	17.50 ± 0.70^{b}
LSD_T	*21.59	*5.44
F-Value	9.25	111.46
P-Value	0.0001	0.0005

Means followed by the same letter in the same column are statistically non-significant ($P < 0.05$); ns = not significant; * = significant of $P > 0.005$
Abbreviations: WS = MacConkey with salt; WOS = MacConkey without salt; PCA = Plate count agar; BP = Baird parker; ND = No data

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All microbial concentrations (10^1, 10^2, 10^3, 10^4 and 10^5) had highly significant ($P < 0.05$) microbial colony count. The significantly high microbial mean count overall for 10^1 concentration was observed in PCA and WS followed by WOS with the least in BP growth media in all farm's growth media (*Table 1*). The highest microbial mean count observed for PCA was in farm 2, and farm 4 followed by farm 5 and farm 1 with the least in farm 3, respectively. The highest microbial mean count observed in farm 2 was 113 $\log_{10}$ cfu/ml and the lowest was 68.50 $\log_{10}$ cfu/ml in farm 3. The highest microbial mean count observed for WS was in farm 4 followed by farm 5 and farm 2 with the least in farm 1 and farm 3, respectively. Farm 4 microbial mean count was the highest and significantly different from the rest of the farms. In WOS the highest microbial mean count was observed in farm 2 with 106.50 $\log_{10}$ cfu/ml and farm 4 followed by farm 1 and farm 3 with the lowest count in farm 5 with 37.50 $\log_{10}$ cfu/ml. Farm 2 and farm 4 microbial mean counts were not significant to each other while farm 1, farm 3 and farm 5 microbial mean counts were not significant to each other.

Farm 4 had the highest BP microbial mean count compared to farm 5, farm 3 and farm 2 with the least observed in farm 1. Farm 4 microbial mean count was significantly different from farm 5, farm 3 and farm 2. The highest microbial mean count from different farms in different concentrations was observed in farm 4 with 113.00 $\log_{10}$ cfu/ml and the lowest microbial mean count was observed in farm 1 BP with 28.00 $\log_{10}$ cfu/ml.

The overall significant microbial mean count for concentration 10^2 was observed in PCA followed by WS and WOS with the least BP in all farms (*Table 1*). The highest microbial mean count observed for PCA was in farm 4 and farm 2 followed by farm 5 and farm 1 with the least in farm 3, respectively. Farm 4 and farm 2 microbial mean counts were not significant to each other. Farm 5 and farm 1 microbial count means were not significant to each other. Farm 1 microbial count mean was significantly different to the farm 3 microbial mean. Farm 4 was observed to have the highest microbial mean count in WS followed by farm 5 and farm 2 with the lowest count in farm 1 and farm 3. Farm 4 was significantly different to farm 5 while farm 2 was significantly different to farm 1. Farm 2 and farm 4 were observed to have the highest microbial count mean for WOS followed by farm 1 with the least count in farm 3 and farm 1. Farm 2 and farm 4 microbial mean counts were significantly different from farm 1. The highest microbial count observed for BP was in farm 4, and farm 5 followed by farm 3 and farm 2 with the least in farm 1.

The highest microbial count mean for PCA was observed in farm 4 and farm 2 followed by farm 1, farm 5 and farm 3, respectively (*Table 1).* Farm 5 and farm 2 microbial mean counts were not significant to each other. Farm 5 microbial count mean was significantly different to farm 3. In WS, the highest microbial count mean was observed in farm 5 and farm 4 followed by farm 2 and farm 3 with the least in farm 1, respectively. In WOS, the highest microbial mean count was observed in farm 2 followed by farm 4, farm 3 and farm 1 with the least in farm 5, respectively. Farm 2 microbial mean count was significantly different to farm 4. The highest microbial mean count observed for BP was in farm 4 and farm 5 followed by farm 3 and farm 2 with the least observed in farm 1. A significant difference was observed between farm 3 and farm 2 microbial mean count.

Farm 2 and farm 3 had the highest microbial mean count for PCA followed by farm 1 with the least difference from farm 3 and farm 5, respectively (*Table 2*). The highest microbial mean count observed in WS was in farm 5 followed by farm 2 and farm 4

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with the least in farm 1 and farm 3. In WOS, farm 2 had the highest microbial count followed by farm 3, farm 4, and farm 1 with the lowest count observed in farm 5, respectively. Farm 2 microbial mean count was significant to farm microbial mean count. Farm 4 had the highest microbial mean count for WS followed by farm 3 with the least in farm 5.

Farm 4 and farm 2 had the highest microbial mean count for PCA followed by farm 5 and farm 3 with the least in farm 1, respectively (*Table 2*). Farm 4 had the highest WS microbial mean count (17.50 $\log_{10}$ cfu/ml) followed by farm 2 with 6.00 $\log_{10}$ cfu/ml. Farm 5 microbial count mean was significantly different from farm 2. Farm 2 was the only farm with a microbial mean count of 14.00 $\log_{10}$ cfu/ml for WOS. Moreover, farm 2 was the only farm with a microbial mean count of 6.50 $\log_{10}$ cfu/ml for BP.

Multivariate data analysis (MVDA)

Multivariate data analysis was applied using a PCA (MVDA) to group correlating microbial count mean. Similar results from ANOVA were obtained from this method (*Fig. 1*). The score plot and loading matrix, based on the first and second principal components (PC1 and PC2) accounted for 86.92% of the total variance. The biplot loading in PC 1 showed that the microbial count means for PCA and WS correlated in different concentration percentages. Plate count agar from farm 4, farm 2 and farm 5 had the highest microbial mean count than other farms and correlates in concentration percentages 2 and 5 including WS microbial count mean from farm 5 and farm 4. All 3 farms had the highest PCA microbial mean followed by WS microbial mean.

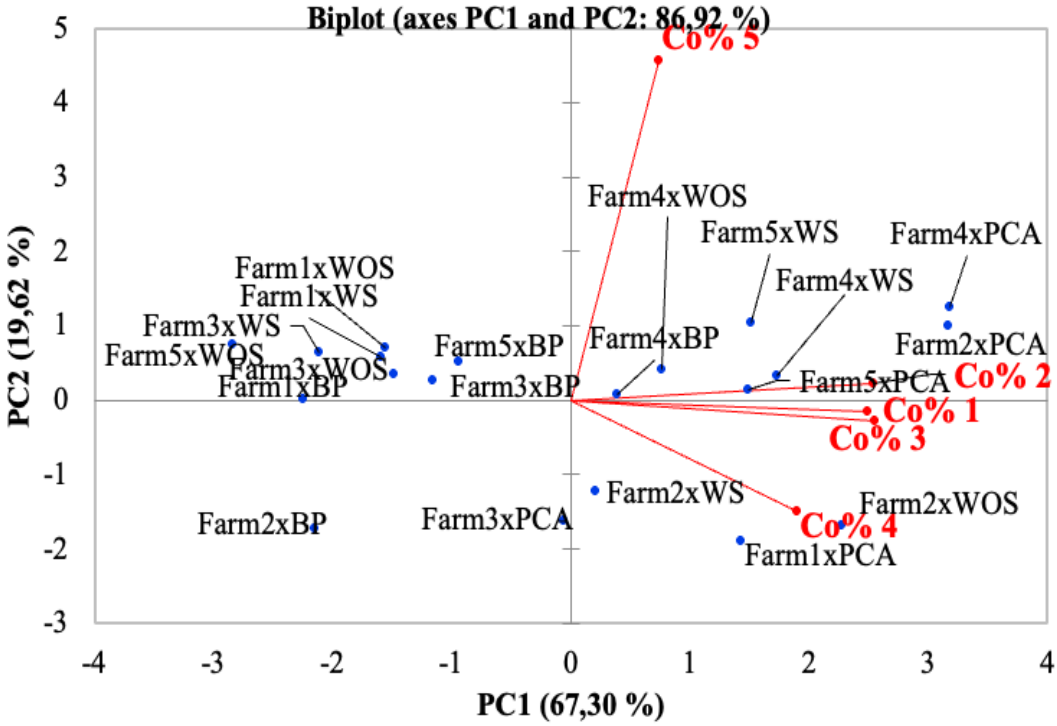

Figure 1. *Principal component biplot illustrating the variations of cabbage microbial count mean correlation in different concentrations in different farms using different growth mediums. WS = MacConkey with salt; WOS = MacConkey without salt; PCA = Plate count agar; BP = Baird parker*

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Spinach phyllosphere microbial count concentrations

Analysis of variance

As illustrated in *Table 3*, five concentrations were utilized to determine the microbial count mean spinach of different farms. A significant interaction between farms and microbial mean counts in the different growth mediums was observed in all concentrations except for 10^2 and 10^5 concentrations (*Table 3*). Microbial concentrations 10^1, 10^3 and 10^4 had highly significant ($P < 0.05$) microbial colony count.

Significant interaction between microbial count and growth medium was observed between farm 4, farm 2, farm 1 and farm 5 (*Table 3*). The highest microbial mean count for PCA was observed in farm 4 and farm 2. Additionally, farm 1 and farm 5 microbial mean counts were not significantly different to each other (*Fig. 2*). The highest microbial mean count for WS was observed in farm 4 and farm 2 followed by farm 5 with the least observed in farm 1, respectively. In WOS, farm 4 had the highest microbial mean count followed by farm 5, with the lowest count observed in farm 2 and farm 1, respectively. Farm 4 microbial mean count was significantly different to farm 5 whereas farm 2 and farm 1 were not significantly different to each other. The highest BP microbial mean count significant difference was observed in farm 5 followed by farm 2 and farm 4 with the least in farm 1. Farm 5 microbial mean count was significantly different to farm 2, farm 4 and farm 1. A significant difference was observed between farm 4 and farm 1 microbial mean count.

Table 3. *Mean log_{10} cfu/ml of bacteria sampled from spinach phyllosphere from different farms*

	Concentrations (log_{10} cfu/ml)				
	10^{-1} Cons	**10^{-2} Cons**	**10^{-3} Cons**	**10^{-4} Cons**	**10^{-5} Cons**
Farms					
Farm 1	67.00 ± 28.19^{b}	55.75 ± 16.88^{b}	32.62 ± 12.19^{b}	10.37 ± 6.90^{c}	5.00 ± 4.24^{b}
Farm 2	92.25 ± 22.68^{a}	$69.00 \pm 23.71^{a,b}$	49.50 ± 19.42^{a}	25.75 ± 15.60^{a}	13.75 ± 14.75^{b}
Farm 4	103.50 ± 30.38^{a}	78.75 ± 24.35^{a}	$41.50 \pm 26.65^{a,b}$	19.37 ± 25.64^{b}	35.00 ± 1.41^{a}
Farm 3	ND	ND	ND	ND	ND
Farm 5	92.37 ± 6.04^{a}	$67.12 \pm 20.44^{a, b}$	44.75 ± 13.54^{a}	16.87 ± 11.63^{b}	7.00 ± 5.45^{b}
LSD_T	*13.54	18.12^{ns}	*11.74	*3.62	*10.47
F-Value	49.45	2.55	6.60	16.48	28.14
P-Value	< .0001	0.10	0.0070	0.0001	
Agar					
WS	99.62 ± 25.17^{a}	76.12 ± 19.91^{a}	49.25 ± 18.17^{a}	17.25 ± 14.69^{a}	6.50 ± 4.65^{b}
WOS	97.62 ± 11.56^{a}	69.25 ± 15.71^{a}	43.75 ± 15.24^{a}	19.00 ± 10.43^{a}	9.00 ± 5.47^{b}
BP	57.75 ± 24.85^{b}	48.37 ± 21.37^{b}	24.25 ± 14.26^{b}	5.12 ± 2.94^{c}	3.75 ± 2.21^{b}
PCA	100.12 ± 15.18^{a}	76.87 ± 21.37^{a}	51.12 ± 17.25^{a}	30.62 ± 22.72^{a}	21.12 ± 16.33^{a}
LSD_T	*13.54	*18.12	*11.74	*3.62	*8.76
F-Value	88.91	5.08	19.71	44.31	16.47
P-Value	< .0001	0.01	< .0001	< .0001	< .0001
Farms x Agar					
LSD_T	*27.08	36.25^{ns}	*23.48	*7.25	13.25^{ns}
F-Value	13.09	1.94	9.76	26.60	15.80
P-Value	< .0001	0.14	0.0003	< .0001	0.30

Means followed by the same letter in the same column are statistically non-significant ($P < 0.05$); ns = not significant; * = significant; ND = No data

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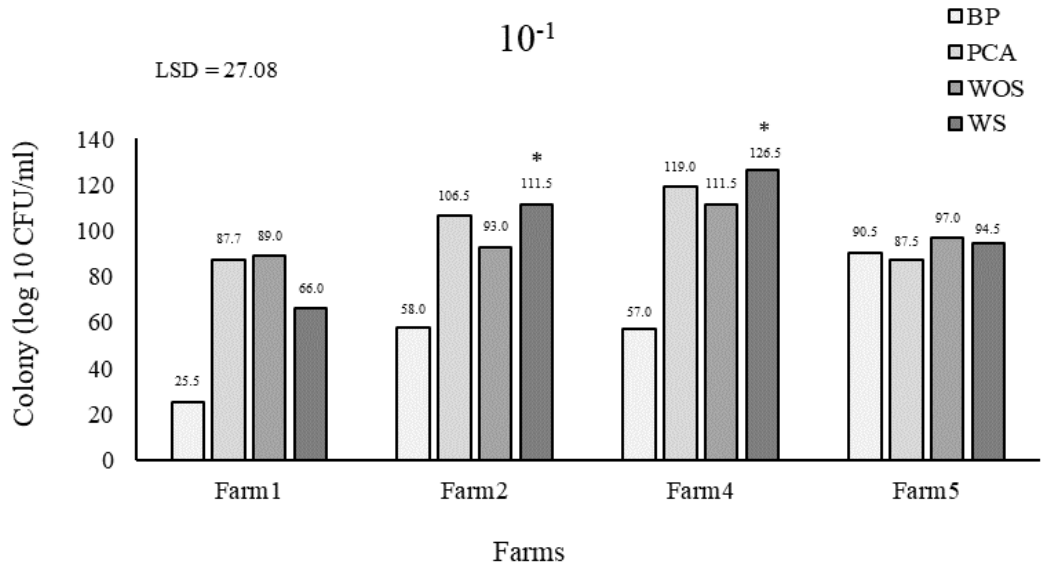

Figure 2. *Microbial mean counts for spinach in this study are presented graphically as mean log_{10} cfu/ml (concentration 10^1). Farm3 had no data. *Interactions of interest for discussion*

The highest microbial mean count for plate count agar was observed in farm 4 and farm 2 followed by farm 5 with the lowest count observed in farm 1, respectively (*Table 3*). Farm 4 and farm 1 microbial mean count for plate count agar were not significant to each other as well as farm 2 and farm 5 microbial mean count. Farm 2 had the highest microbial mean count for WS followed by farm 4 and farm 5 with the least observed in farm 1, respectively. Farm 2 microbial mean count was significant to farm 4 and farm 5. Farm 5 and farm 1 were significantly different from each other. In WOS, farm 5 and farm 1 had the highest microbial mean count followed by farm 4 and farm 2, respectively. Farm 4 had the highest microbial mean count for BP followed by farm 5, and farm 2 with the least observed in farm 1.

The highest plate count agar microbial mean count was observed in farm 4 followed by farm 2 and farm 1 with the least observed in farm 5, respectively (*Table 3; Fig. 3*). Farm 4 was significantly different to farm 2 microbial mean count. Farm 2 was observed with the highest microbial mean count in WS followed by farm 4 and farm 5 with the least in farm 1, respectively. Farm 2 farm microbial mean count was significantly different to farm 4 microbial mean count. A significant growth was also observed in WOS with the highest microbial mean count in farm 5 followed by farm 1 and the lowest count observed in farm 4 including farm 2. Farm 5 was significantly different to farm 1. In BP, M farm 4 had the highest microbial mean count followed by farm 5 with the least count observed in farm 2 and farm 1 microbial mean count.

Farm 4 had the highest microbial mean count for PCA followed by farm 2 with the lowest count observed in farm 5 and farm 1, respectively (*Table 3; Fig. 4*). Farm 4 microbial mean count for PCA was significantly different to farm 2. Farm 2 microbial mean count was significantly different to farm 5 and farm 1. Farm 5 had the highest microbial mean count in WOS followed by farm 1 and farm 2 with the lowest count observed in farm 4. Farm 5 microbial mean count was significantly different to farm 1 microbial mean count. The farm 2 was observed with the highest microbial mean count in WS followed by farm 4 with the lowest count in farm 1 and farm 5. Farm 4 microbial mean count was significantly different to farm 1, farm 2 and farm 5 microbial mean

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count. Farm 5 had the highest microbial mean count for BP, followed by farm 2 and farm 1 with the lowest count observed in farm 4, respectively. Farm 5 microbial mean count was significantly different to farm 1 and farm 4.

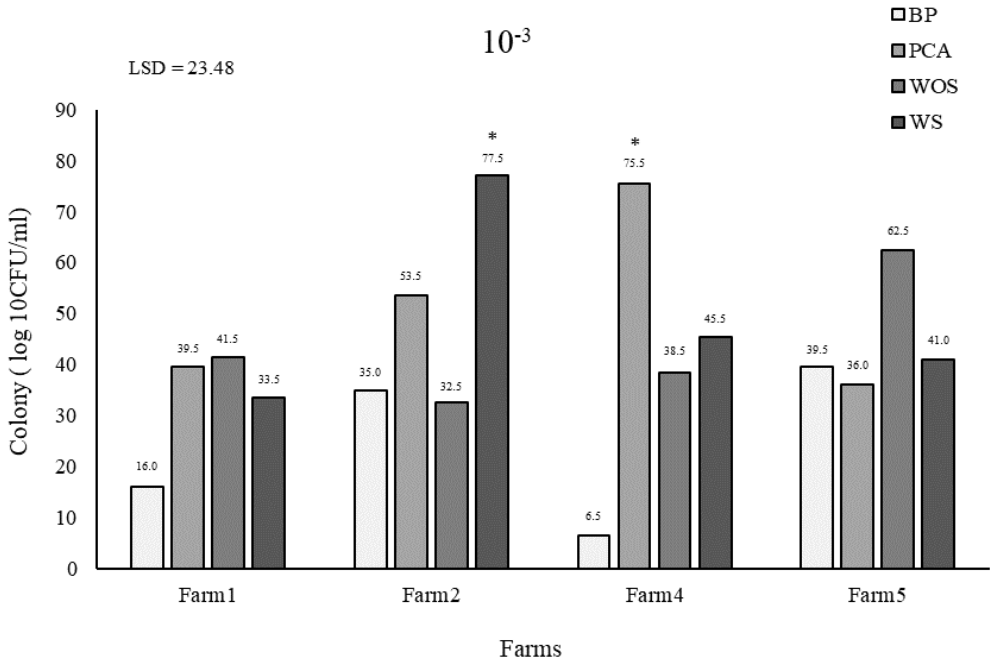

Figure 3. *Microbial mean counts for spinach in this study are presented graphically as mean log_{10} cfu/ml (concentration 10^3). Farm3 had no data. *Interactions of interest for discussion*

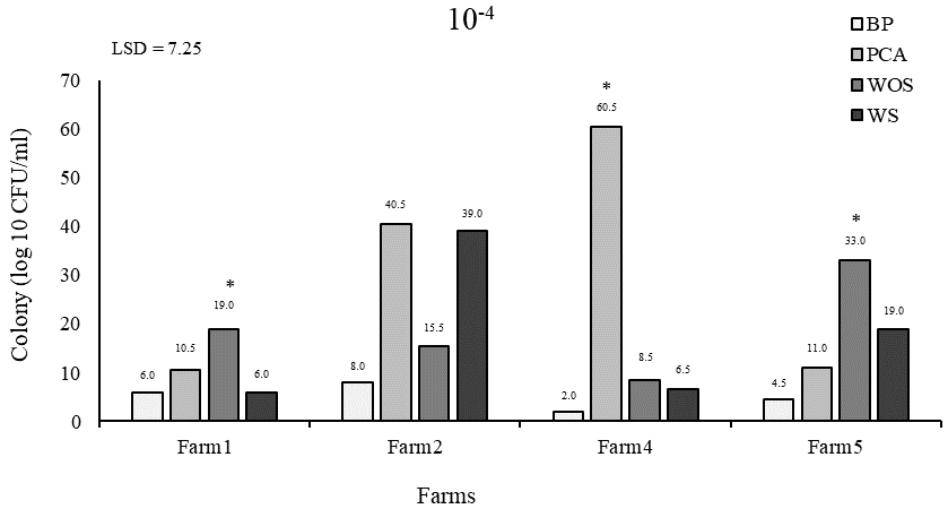

Figure 4. *Microbial mean counts for spinach in this study are presented graphically as mean log_{10} cfu/ml (concentration 10^4). Farm3 had no data. *Interactions of interest for discussion*

In PCA, farm 2 and farm 4 had the highest microbial mean count followed by farm 1 and farm 5, respectively (*Table 3*). Farm 4 and farm 1 microbial mean count were significantly different to each other as well as farm 4 and farm 2 microbial mean count. In WS, farm 2 had the highest microbial count mean followed by farm 5 and the least was

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observed in farm 1 and farm 4, respectively. Farm 5 had the highest microbial count for WOS followed by farm 1 with the least in farm 2 and farm 4, respectively. In BP, farm 2 had the highest followed by farm 1 wit the least in farm 4 and farm 5, respectively.

Multivariate data analysis

Multivariate data analysis was applied using a PCA to group correlating microbial count mean. Similar results from ANOVA were obtained from this method (*Fig. 5*). The score plot and loading matrix, based on the first and second principal components (PC1 and PC2) accounted for 81.72% of the total variance. The biplot loading in PC 1 (61.44%) showed that the microbial mean count for PCA and WOS correlated in concentration percentages 2. 4 and 5. Plate count agar from farm 2 and farm 4 microbial mean count correlates and is significant as these two farms had the highest microbial mean count followed by farm 3. Farm 1 WOS microbial mean count and farm 3 were significant. On the other hand, farm 2, farm 4 and farm 5 WS microbial mean count showed a correlation in concentration percentages 1 and 3 due to high microbial mean count.

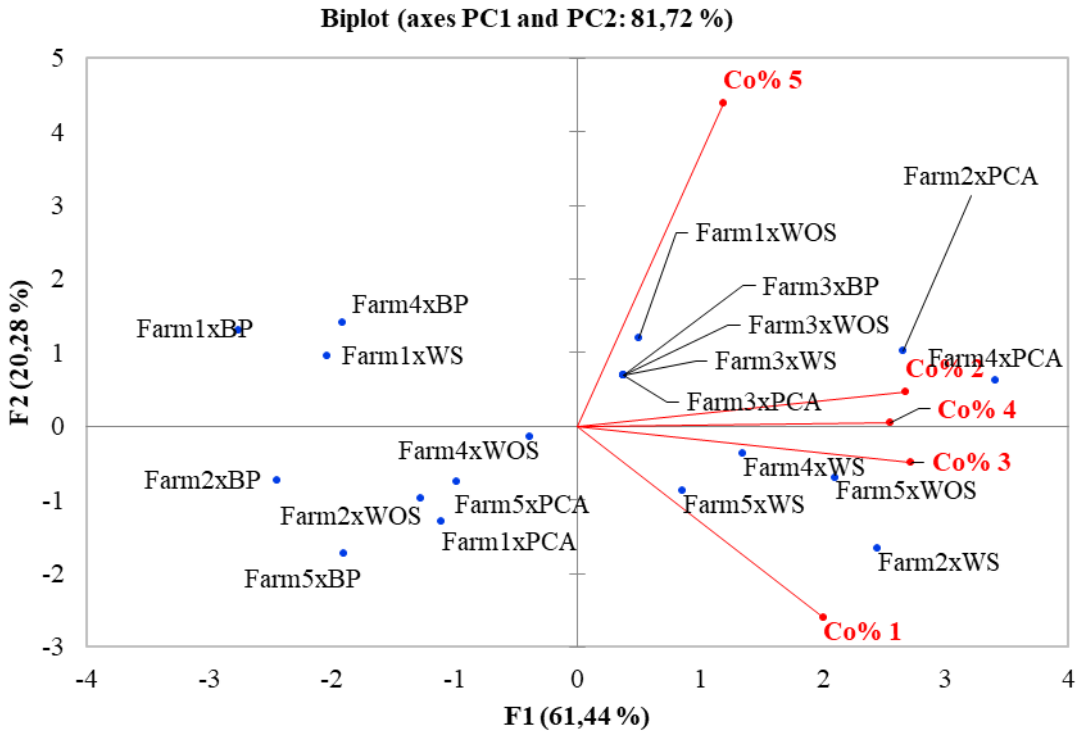

Figure 5. *Principal component biplot illustrating the variations of spinach microbial mean count correlation in different concentrations in different farms using different growth media. WS = MacConkey with salt; WOS = MacConkey without salt; PCA = Plate count agar; BP = Baird parker*

Bacterial contamination from various farms

Cabbage phyllosphere microorganisms

In this present study, farm 2, farm 3 and farm 4 had the highest number of pathogens identified for cabbage followed by farm 5 with the least number observed in farm 1.

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(*Table 4*). With regards to spinach, farm 2 had the highest number of identified pathogens followed by farm 4 with the least number observed in farm 1 and farm 5.

Table 4. *Identification of pathogens identified from spinach and cabbage phyllosphere isolates*

	Farms	**Pathogens identified**
Cabbage phyllosphere	Farm 1	*Pseudomonas luteola, Serratia ficaria*
	Farm 2	*Brevundimonas vesicularis, E. coli, Chryseomonas luteola* (93.9%), *Staphylococcus lentus, Staphylococcus xylosus, Proteus mirabilis*
	Farm 3	*Staphylococcus sciuri* (76.1%) with second taxon *Staphylococcus xylosus* (23.8%) and third, *Staphylococcus lentus* (0.1%), *Serratia liquefaciens, Pseudomona luteola*
	Farm 4	*Acinetobacter Baumanni, Staphylococcus aureus* (97.7%), *Staphylococcus epidermis* (79.4%), next taxon was *Staphylococcus aureus* with (18.4%), *Burkholderia cepacia, E. coli*
	Farm 5	*Yersinia enterocolitica* (99.8%) next taxon *E. coli* (0.1%), *Staphylococcus aureus*
Spinach phyllosphere	Farm 1	*Staphylococcus aureus, Pseudomonas stutzeri, E. coli, Serratia ficaria* (97.0%)
	Farm 2	*Brevundimonas vesicularis, Burkholderia cepacia, Pseudomonas stutzeri Staphylococcus aureus, Pseudomonas luteola, E. coli, Pseudomonas aeruginosa, Citrobacter freundii, Serratia marcescens, Morganella morganii, Listeria monocytogenes, Listeria ivanovii*
	Farm 3	No data
	Farm 4	*Staphylococcus sciuri, Proteus mirabilis, E. coli, Yersinia enterocolitica, Proteus penneri* (99.6%), next taxon *Proteus Vulgaris group* (0.2%), *Providentia stuartii, Citrobacter freundii, Listeria ivanovii*
	Farm 5	*Stenotrophomonas maltophilia, Staphylococcus heamolyticus* (85.9%)

(%) – viable specie count shown by API Web

In this present study, farm 1 cabbage was contaminated with *Pseudomonas luteola,* and *Serratia ficaria.* These pathogens are versatile gram-negative bacteria that mainly emante from soil, water and living organisms including animals, insects and human. The possibility of contamination may be from personnel through inadequate personnel hygiene during sorting out of produce before packaging as it is hand sorting by workers. The farm posses the food safety programs including several produce safety guide which are in place. Revision of hygiene and sanitation standards, frequent monitoring is required frequently to avoid negligence of personnel regarding hygiene practice. There are no similar cases in the literature to support this hypothesis. In this present study, cabbage obtained from farm 2 had the highest number of microbes from various taxa than other farms followed by farm 4. A great possibility exists that microbial diversity from farm 2 is influenced by poor infrastructure including poor hygiene practices since it was observed that the farm was not in good conditions from the field, packaging to vehicle. *Staphylococcus* spp. and *E. coli* from farm 2 may be due to hand preparation from harvesting to storage without proper disinfection between processing. The growers guide and food safety and quality assurance standards in place were neglected due to high demand production of cabbage so the

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farmer ended up focusing more on production rather than food safety. A strict supervision including frequent produce safety rule which is coupled by critical control points is required. The present study concluded that the prevalence of *Proteus mirabilis* from farm 2 and farm 4 was attributed to non-composted manure contaminating the vegetables, as livestock manure are utilized as fertilizer. Farm 4 does not only produce leafy greens but also produce livestock. The time that manure are applied as fertilizer to produce and the harvest time is critical in order to avoid contamination of crops.

The presence of *Serratia liquefaciens* obtained in farm 3 indicate contamination from the environment and personnel as this species is considered a human pathogen. Farm 3 utilizes conveyer belt where personnel sort out, peel and cut defect by hands. Contamination can move from one batch of produce to the other. In addition, *staphylococcus* species is utilized as hygiene quality indicator where its presence indicated inadequate hygiene practices. Pasewu et al. (2014) highlighted contamination of cabbage in the following order, *Staphylococcus aureus* (51%), *E. coli* (28%) and *Pseudomonas aeruginosa* (4%). The bacterial load identified on leafy vegetables increase with time during storage (Söderqvist et al., 2017).

Farm 4 vegetable contamination could be through water and livestock present around the farm. Another possibility from farm 4 can be the utilization of animal-based fertilizer for the enrichment of soil which contributes to soil contamination leading to contamination of crops. With regards to farm 4, the microbial pathogens identified were mostly from the *Staphylococcaceae* family therefore suggesting poor hygiene practices from personnel followed by *E. coli* suggesting fecal contamination which might be due to various livestock on the farm. It has also been emphasized by Slater et al. (2018) that *Staphylococcus aureus* isolated from the leafy green samples was an indication of poor hygienic practices by farmers.

Different *Acinetobacter* species are generally associated with various habitats such as soil, water, sewage, humans, foods, and animals and have been involved in a variety of nosocomial infections, including bacteraemia, urinary tract infection and secondary meningitis (Almasaudi, 2018). Hamouda et al. (2011) isolated *Acinetobacter baumanii* species from fecal specimens, skin, nostril and ear swabs from pigs and cattle slaughtered for human consumption from a list of about 3111 farms. *Acinetobacter baumanii* was also isolated from farm 4 and this is indicative of insufficient hygiene during processing and a possible cross-contamination from livestock. Possibility being contamination from livestock around the farm. It is therefore important that operating procedures should be followed strictly particularly with regards to hand washing facilities to prevent contamination as these pathogens are present where there is poor hygiene practices and fecal contamination.

In this study *Y. enterocolitica* was isolated from farm 5 indicate a possibility of crops growing closer to soil leading to contamination of cabbage. Thorough washing of leafy greens is required to eliminate or reduce microbial load from produce. Since well the farm did not have any livestock present, the present study conclude contamination from the soil and a possible cross-contamination during washing of produce. *Yersinia enterocolitica*, *Serratia* spp. occur naturally in soil and water and *Yersinia* infections have overtaken *Shigella* and *Salmonella* species as the most common cause of bacterial gastroenteritis (Aziz and Yelamanchili, 2018). Additionally, *Y. enterocolitica* pathogen has been isolated from a variety of animals with pigs being the most common source and the spread can be from one pig to another in a herd. *Yersinia enterocolitica* has also

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been found to infiltrate into plants such as cabbage, peas (*Pisum sativum* var. s*accharatum*) and oats (*Aneva sativa*) from infected soil and water as its survival is affected by moist environmental conditions (Vlu et al., 1991). *Yersinia enterocolitica* was more frequently detected in ready-to-eat vegetables with the highest prevalence observed in Finland where it was isolated from 33% of fresh leafy vegetables (Verbikova et al., 2018). Furthermore, between 2006 and 2009 approximately 7,600 and 9,000 cases of yersiniosis were reported from Europe annually and WHO registered 340 deaths between 1994 – 2008.

Spinach phyllosphere microorganisms

With regards to farm 1, *Staphylococcus* spp. and *Pseudomonas* spp. were predominant which generally suggests poor hygiene which contributed to microbial load. Personnel hygiene and proper sanitation are important. Proliferation of these isolated organisms is possible with a possibility of contamination. Proper sanitation ad hygiene from personnel can curb the contamination to avoid any infection.

Listeria spp. was detected in farm 2 and farm 4 contamination may emanate from poor agricultural management and inadequate hygiene during pre-harvest and post-harvest processes. Farm 4 *Listeria ivanovii* may be contaminated by livestock as *Listeria* emanate from the environment and intestinal tract of domestic and livestock and is shed in feces. In South-West Nigeria, *Pseudomonas* species were isolated from both irrigation water and vegetables, the data indicate that contamination of the vegetables was increased because of contaminated water utilized for irrigation (Akinde et al., 2016). In this study, *Pseudomona stutzeri* was isolated from farm 2 and farm 1. Radovanovic et al. (2020) demonstrated that the prevalence, persistence, and ability of *Pseudomonads* to form biofilm on surfaces of food processing plants enhances their resistance to adverse conditions including several antimicrobial treatments during washing. according to Mritujay and Kumar (2017) spinach samples isolated had the highest microbial count mean of 7.3 log cfu/g with a frequency of 6.1-9.6 log cfu/g than cabbage and cucumber (*Cucumis sativus*) thus indicating poor handling of spinach during storage. It is also shown that the prevalence of microorganisms particularly in sprouts (*Brassica oleracea* var. *gemmifera*) and fresh spinach can be significantly higher at the final post-harvest stages compared to the early stages of handling (Frank et al., 2011). This may be due to subsequent recontamination including pathogen amplification during postharvest activities of minimal processing. Bacteria can retain or be trapped in plant parts even after vigorous disinfection (Solomon et al., 2002). This simply demonstrates the ability of a pathogen to utilize its ability to resist certain disinfectants and internalize during the washing of fresh leafy vegetables following recontamination.

Citrobacter spp. are facultative anaerobic, motile, gram-negative bacilli in the *Enterobacteriaceae* family that is widely distributed in the environment and intestinal tracts of human and animals (Murray et al., 2010; Adegun et al., 2019). Sixty-six bacteria were isolated from 60 vegetable samples, of these isolates, *Salmonella* spp recorded 43.3%, followed by *Citrobacter freundii* 18.3%, *Klebsiella* spp. 15.0%, *Enterobacter* spp. 11.7%, *Proteus* spp. and *Alcaligenes* spp. 5.0% each, *E. coli* and *Providencia* spp. 3.3% each and *Vibrio* spp. 1.7% (Oluboyo et al., 2019). Another study isolated and characterized *Citrobacter* species in fruits and vegetables sold for consumption in ILE-IFE, Nigeria and concluded that *Citrobacter* spp. recovered from fruits and vegetables are not flora to fruits and vegetables but are frequently isolated from animals and as an

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opportunistic pathogen in human. In this present study, the contamination of spinach likely emanates from fecal contamination from animals or inadequate hygiene from handling of produce during cutting to a specific size before storage.

Proteus mirabilis was isolated from spinach and tomato from two different local vegetable agricultural fields (Shoket et al., 2014). *Proteus* spp. has growth potential even at low infectious doses and are potential human health risk most commonly causing urinary tract infections and infection-related kidney stones (Scherberich et al., 2021). In this study, *Proteus mirabilis* may indicate poor agricultural practices due to poor harvesting practices. Regular surveillance or analysis of food safety and critical control points is necessary to avoid contamination from the field to the minimal processing establishment.

With regards to farm 4, *Staphylococcus* spp. were the most predominant organisms isolated from spinach. The presence of *Staphylococcus* spp. could be a possibility of poor hygiene and sanitation around the farm. *Pseudomonas* spp. was also isolated, and its presence could be due to their tolerance strategies to survive under certain temperatures and environments and conducive conditions of proliferation due to improper storage of spinach after sorting. Control of operation, maintenance and sanitation including personal hygiene must be maintained. Strict measure must be implemented about the storage of spinach and cabbage to avoid temperature abuse which enable organisms to utilize tolerance strategies even forming biofilm to survive and thrive.

Providencia species have been commonly found in soil and sewage and have been broadly isolated from chickens (*Gallus domesticus* L.), cows (*Bos taurus* L.), and dogs (*Canis familiaris* L.) (Wie, 2015). *Providencia* infections include urinary tract, gastroenteritis, and bacteraemia, and infections are usually nosocomial. *Providencia* spp. represent an emerging problem because of the increasing prevalence of antibiotic resistance secondary to extended-spectrum beta-lactamase. The genus *Providencia* spp. found in farm 5 is likely to emanate from contamination of soil, water utilized for irrigation and inadequately treated sludge utilized as fertilizer to the crops. The microbial status of water utilized for the irrigation of fresh leafy greens needs to be prioritized to avoid the uptake and contamination of bacteria into vegetables. Sludge or manure-rich soil influences microbiological and chemical parts of soil and vegetables. The genus *Providencia* spp. found in farm 4 is likely to emanate from contaminated soil as animal manure is utilized as fertilizer. Infection and illness can be caused by an extremely low dosage of toxins. The farm also utilizes basin to wash produce and in-between washing, the water is not changed frequently and the knives for cutting and trimming produce are not been disinfectant to avoid cross-contamination particularly when more batches of spinach are to be washed.

Stenotrophomonas maltophilia emanates from isolated from manure, chicken feces, soil, plants, salads, water, and raw milk and has been implicated as the causative pathogen in respiratory tract infections, endocarditis, bacteraemia, meningitis, and urinary tract infections. Pre-packaged ready-to-eat salads washed with chlorinated water before sale showed insufficient to remove *S. maltophilia* from these items, possibly because the bacterium may exist in biofilms (Qureshi et al., 2005; Agri et al., 2022). In this present study, it is assumed that *Stenotrophomonas maltophilia* from spinach isolates may be contaminated by manure or possible contamination from the soil. For this reason, it is important to the time for the application of manure and the time to harvest to avoid contamination.

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Conclusion

The incidence and predominance of microbial pathogens in spinach and cabbage in sampled farms is due to inadequate hygiene during processing. Bacteria more easily attach and colonize vegetable surfaces with grooves than those with smooth surfaces (Warning and Datta, 2017). Cabbage has the largest surface area compared to spinach, making it prevalent in *Enterobacteriaceae*. In this present study, it is justifiable to conclude that cabbage has a large surface area compared with spinach, which enables ease of attachment, internalization and colonization of various microorganisms. Variations of bacterial pathogens and their prevalence could be attributed to the differences in minimal processing infrastructure which includes inadequate hygiene, poor agricultural practices and negligence. In general, the presence of predominant microorganisms indicates poor extensive human, unhygienic conditions contribute to a succession of microorganisms. Results in this present study further corroborated that cabbage from various farms harbored diverse bacterial communities, and the communities from each farm on each cabbage were significantly distinct from one another according to the bacterial family except for *Staphylococcus* species and *E. coli* predominance. Therefore, it is recommended that these vegetables be thoroughly washed before any consumption especially when consumed uncooked, specifically in green salad.

In 1999 and 2010 studies reported that *L. ivanovii* was exclusively linked to ruminants but it was later highlighted that *L. ivanovii* infections occurred in humans after the ingestion of foodstuffs that was contaminated. It was concluded that a wide variety of foodstuffs are now a source of this pathogen and that, similarly to *L. monocytogenes, L. ivanovii* is capable of persistence in food production establishments (Rossi et al., 2022). *Listeria* pathogen is difficult to eliminate, particularly from the food chain including ready-to-eat foods and vegetables. Persistence for *Listeria* strains is contributed by extrinsic factors including poor hygiene and ineffective sanitizer and the presence of specific genes responsible for biofilm formation (Lee et al., 2019).

It is concluded that the bacterial contamination frequency and degree in this present study were significantly considerable. The knowledge of the composition and diversity of *Enterobacteriaceae* and *Staphylococcaceae* communities in cabbage and spinach may be useful in the establishment of control measures to mitigate the transmission of pathogens to consumers. Food handlers need to be repeatedly reminded of hygiene and food safety. Monitoring and regular supervision are essential to control and minimize microbial hazards that leads to contamination.

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