MICROBIAL ANALYSIS OF READY TO EAT LEAFY VEGETABLES COLLECTED FROM VARIOUS AREAS OF DISTRICT SWAT, KHYBER PAKHTUNKHWA

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ABSTRACT

The contamination of leafy vegetables with microbes and heavy metal toxicity is one of the world's most serious environmental issues. Despite the fact that leafy vegetables are well known around the world for their many health benefits. A study was done to determine the microbiological condition of leafy vegetables including Lettuce, Cabbage, Coriander and Mint from different Swat regions (Matta, Khwazakhela, Mingora and Kabal). The study was conducted in the Environmental Horticulture Laboratory at the University of Agriculture Peshawar, Department of Horticulture during the 2018-19 academic year. The experiment was designed using the RCBD method, with one factor and three replicates. Lettuce, Cabbage, Coriander and Mint were found to have the highest bacterial group maximum range for TBC (5.58, 5.56, 5.52 and 5.49 log cfu g^{-1}), Enterobacteriaceae (5.49, 5.46, 5.43 and 5.41 log cfu g^{-1}), *E. coli* (4.08, 4.04, 4.13 and 4.03 log cfu g^{-1}) *Salmonella* (4.05, 4.03, 4.04 and 4.02 log cfu g^{-1}) and *Listeria* (4.01, 3.71. 3.94 and 3.79 log cfu g^{-1}) was recorded for the vegetable's samples collected from Mingora location while lowest values of Lettuce, Cabbage, Coriander and Mint for TBC (4.70, 4.66, 4.61 and 4.61 log cfu g^{-1}), Enterobacteriacae (4.61, 4.56, 4.54 and 4.53 log cfu g^{-1}), *E. Coli* (3.70, 3.51, 3.61 and 3.53 log cfu g^{-1}), *Salmonella* (3.60, 3.55, 3.57 and 3.51 log cfu g^{-1}) and *Listeria* (3.45, 3.15, 3.35 and 3.25 log cfu g^{-1}) was getting for Matta location. The conclusion was reached that the bacterial groups were most prevalent in the vegetables gathered from the Mingora region since all the microorganisms analyzed exhibited a significant difference.

Keywords: leafy vegetables, Various location, Bacterial groups.

INTRODUCTION

Fresh produce contamination is becoming a significant global food safety problem. Leafy vegetables are well known for their many health advantages all around the world. These veggies are becoming more popular, especially in metropolitan areas and are high in calcium iron, folic acids, vitamin A C, dietary fiber and other minerals. The microbiota of leafy green

vegetables is varied and can contain pathogen or potential pathogen leafy green vegetables are very sensitive to bacterial contamination and multiplication. These bacteria can have transferred to plant from various sources like soil, manure, irrigation water, equipment and other human anthropogenic activities (Pinter *et al*., 2013). However leafy green vegetables (LGV) are frequently linked with various microorganisms which are directly responsible to food borne illnesses and outbreak in human beings throughout the world. Particularly these leafy green vegetables (LGV) or their Ready to Eat (RTEs) salads are frequently consumed raw or with minimal processing or no further treatment (Ilic *et al*., 2012). The United States center for Diseases control and prevention center (CDC) reported that the number of outbreaks associated with fresh produce consumption has been increased prominently during the past decade (1995 – 2005) (Jhonson *et al*., 2005).

METHODOLOGY

Vegetables sullied with different toxins can cause wellbeing issues all over the world. To screen the status of new utilized verdant vegetables, a consider titled "Microbial analyses of ready to eat leafy vegetables collected from various areas of district Swat, Khyber Pakhtunkhwa" was conducted in 2019 at the Environmental Horticulture Laboratory, The University of Agriculture Peshawar (UAP). The experiment was designed using a Randomized Complete Block Design (RCBD) with the factor of different locations (Matta, Khwazakhela, Kabal, Mingora) and vegetables includes Lettuce, Cabbage, Coriander and Mint that were considered and replicated three times.

Samples Collection

Various leafy vegetables consumed fresh, such as lettuce, mint, coriander, and cabbage, were collected from various areas of Swat valley. Plant samples were brought to the UAP Environmental Horticulture Laboratory and tested for the presence of microorganisms (food borne pathogens). For the presence of microbes, sterile polythene bags were used to collect vegetable samples. After that, 25g samples of each vegetable were separated and analyzed for the presence of microbes (Feroz *et al.,* 2013).

Laboratory Procedure:

Materials and Equipment Sterilization:

To prevent contamination all glassware's used for microbial analyses was sterilized under laboratory conditions through standard procedure with tap water washing properly fallowed

by distilled water and autoclaving them at 121 ^{o}C for 20 minutes at 15 pound-force per square inch (psi).

Media Preparation

All the media was prepared according to their prescribed standards and protocol. The media in powdered form were prepared in distilled water with their specific amount and weight using the conical flask. Heater with magnetic stirrer was used for heating and stirring the media when needed, autoclaving of media was performed where needed. After the media cooled down it was poured to the sterile plates in Laminar Flow Unit under sterile conditions. Different culture media were prepared for various microorganisms as mention in table below.

Microbial Analyses

Various vegetable samples were kept in cooler at 4 ±2^{o}C. Vegetables will be separated to weigh 25 g from each sample. 50 ml liquid NaCl was added to sterile polythene bags containing 25 g sample, followed by hand shaking for 2 minutes so as to detach microbes from vegetable samples. Then the rinsed water from samples was poured in sterile glass tubes and one tenth serial dilution of samples was made before analysis using NaCl (0.085%). Fifty (50) µl from the determined diluted samples were taken and inoculated on selected media in plates. Finally, the media containing colonies were incubated for specific time and temperature in the incubator as shown in table below.

Microbial media, their incubation time and temperature for specific microorganism.

Media	**Incubation Time**	**Incubation Temperature**	**Microorganisms**
Tryptic Soy Agar (TSA)	18 – 24 hrs.	24± 2^{o}C	Total Bacterial Count
Violet Red Blue Dextrose agar (VRBD)	24 hrs.	37± 2^{o}C	*Enterobacteriaceae, E. coli Salmonella, Klebsiella, Shigella* etc.
E. Coli (EC Broth)	18 – 24 hrs.	37± 2^{o}C	*E. coli, Bacillus subtilis, Aeruginosa, Enterobacter, Klebsiella*etc.
Salmonella shigella agar (SS Agar)	18 – 24 hrs.	37 ± 2 ^{o}C	*Salmonella* and some *Shigella* spp.
Agar Listeria	16 – 24 hrs.	20 – 28 ^{o}C	*Listeria* spp.

The plates after incubation were analyzed for the following groups of bacteria.

- ✓ Total bacterial count
- ✓ Enterobacteriaceae
- ✓ *Escherichia coli*
- ✓ Salmonella sp.
- ✓ Listeria sp.

Data Analysis

Data were recorded on the Statistics 8.1 software and the means were computed with the help of LSD test (Steel *et al*., 1997).

RESULTS AND DISCUSSION

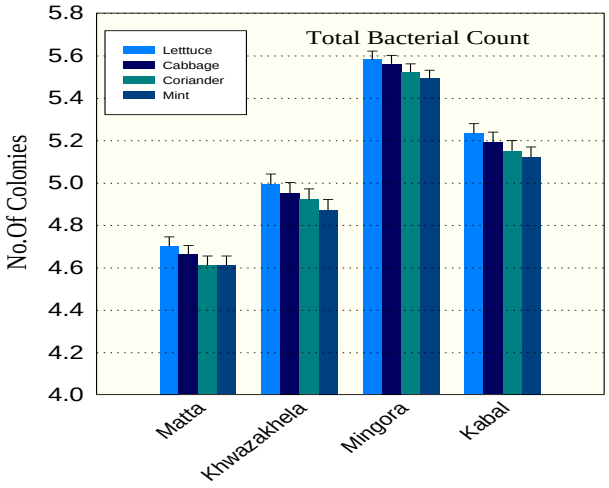

Figure.1: Total bacterial count (log cfu g^{-1}) in Lettuce, Cabbage, Coriander and Mint collected from various locations of district Swat.

Total bacterial count:

The data statistical analysis revealed a significant variation for total bacterial count among all the locations (Fig. 1 or Table 1). Maximum TBC for the crops as lettuce (5.58 log cfu g^{-1}), cabbage (5.56 log cfu g^{-1}), coriander (5.52 log cfu g^{-1}) and mint (5.49 log cfu g^{-1}) was recorded for the vegetables sample collected from Mingora region, followed by Kabal region lettuce (5.23 log cfu g^{-1}), cabbage (5.19 log cfu g^{-1}), coriander (5.15 log cfu g^{-1}) and mint (5.12 log cfu g^{-1}) whereas the minimum TBC for lettuce (4.70 log cfu g^{-1}), cabbage (4.66 log cfu g^{-1}), coriander (4.61 log cfu g^{-1}) and mint (4.61 log cfu g^{-1}) was getting for the vegetables sample collected from Matta region. Vegetables get contaminated with several

microorganisms due to various reasons like the use of unhygienic water for irrigation, raw sewage, manure fertilizer, during harvesting etc. and usually cause different diseases (Razzaq *et al.*, 2014; Brackett and Splittstoesser, 1992). When the results of the studied vegetables plant samples for total bacterial count were compared, a significant difference was found among all the locations. The high range for TBC was found in Mingora region, which may be due to Mingora region being the most warm and contaminated spot as compared to other regions farmer use waste water and other sludge, from unclean water, from soil, and so on. According to Izumi *et al.* (2004), the increased occurrence of various microorganisms is due to polluted environmental conditions and microbes present in waste water and soil.

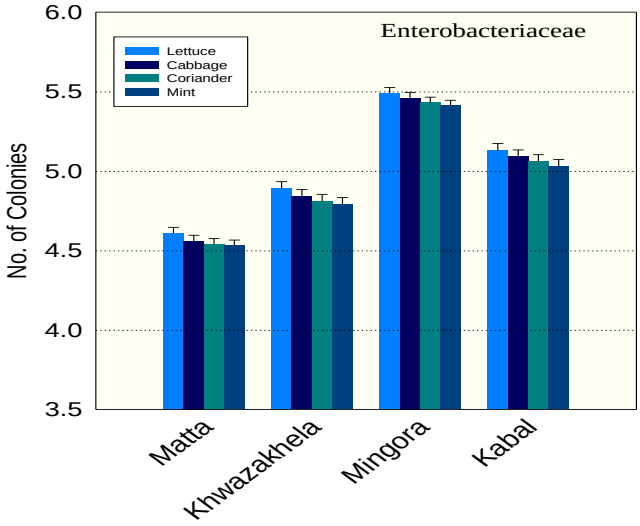

Figure. 2: Enterobacteriaceae (log cfu g^{-1}) in Lettuce, Cabbage, Coriander and Mint collected from various locations of district Swat.

Enterobacteriaceae:

The statistical analysis of the data showed a significant difference among all the selected spots (Fig. 2 or Table 2). Regarding the means maximum enterobacteriaceae for lettuce (5.49 log cfu g^{-1}), cabbage (5.46 log cfu g^{-1}), coriander (5.43 log cfu g^{-1}) and mint (5.41 log cfu g^{-1}) followed by Kabal region lettuce (5.13 log cfu g^{-1}), cabbage (5.09 log cfu g^{-1}), coriander (5.06 log cfu g^{-1}) and mint (5.03 log cfu g^{-1}) while minimum values lettuce (4.61 log cfu g^{-1}), cabbage (4.56 log cfu g^{-1}), coriander (4.54 log cfu g^{-1}) and mint (4.53 log cfu g^{-1}) was recorded for the vegetables samples collected from Matta spots. Human pathogenic bacteria are present in large amount in salad vegetable which is a potential risk to human and resistant antibiotic is growing more and more. It may be due to polluted water used for irrigation, sewage sludge mostly used as fertilizer, improper and contaminated harvesting (Olayemi, 1997). A major change was observed for enterobacteriaceae in all vegetable's samples on the

basis of various spots high number of enterobacteriaceae was found in Mingor region it may be due to Mingora area is the hot and contaminated area among the other studied locations. Mostly people use these waste water and other municipal waste in the field which leads to microbe's occurrence. Mangoli and Manani (2014) stated that high range of enteriobacteriaceae in vegetables is due to waste water pathogens, high temperature and fecal pollutants.

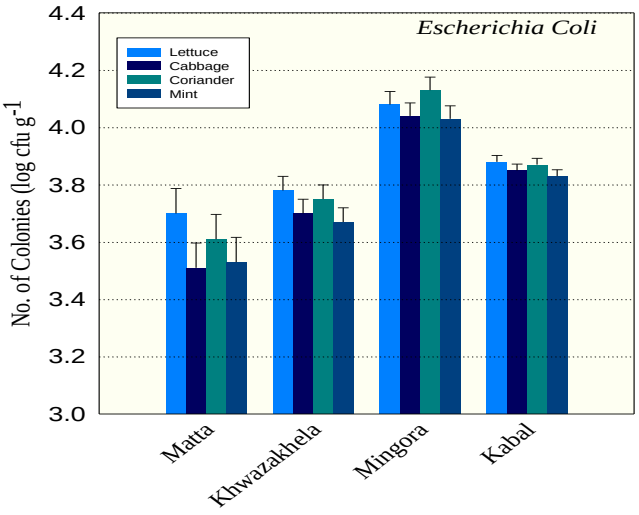

Figure. 3: *Escherichia coli* (log cfu g^{-1}) in Lettuce, Cabbage, Coriander and Mint collected from various locations of district Swat.

***Escherichia coli*:**

Data pertaining for *Escherichia coli* shows a significant variation between all the regions (Fig. 3 or Table 3). It is declare from the data that the upper limit for lettuce (4.08 log cfu g^{-1}), cabbage (4.04 log cfu g^{-1}), coriander (4.08 log cfu g^{-1}) and mint (4.03 log cfu g^{-1}) was recorded for the samples collected from Mingora locality followed by Kabal region lettuce (3.88 log cfu g^{-1}), cabbage (3.85 log cfu g^{-1}), coriander (3.87 log cfu g^{-1}) and mint (3.83 log cfu g^{-1}) while lower limit for lettuce (3.70 log cfu g^{-1}), cabbage (3.51 log cfu g^{-1}), coriander (3.61 log cfu g^{-1}) and mint (3.53 log cfu g^{-1}) was recorded for the vegetables samples collected from Matta region. The vegetables are vulnerable to pathogen attack at all stages of crops cultivation and harvest these vegetables and fruit can get contaminated by different pathogenic microorganism, human diseases originated due to contaminated vegetables consumption and had been reported by many countries of the world (De Rover, 1998; Anonymous, 2013). In our observation high number of *E. coli* was noted for the vegetables plant samples collected from Mingora region it might be due to Mingora is the most populated, industrialized and warm place as compared to other studied location. Beuchat,

(1996); De Rover, (1998) stated that microorganisms present in irrigation water, urban wastes manure and soil its survival depend upon on sunlight, relative moisture, rainfall etc.

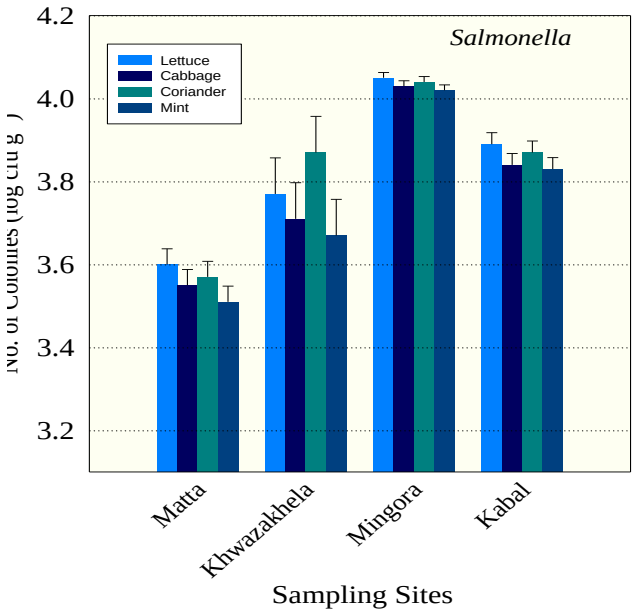

Figure. 4: *Salmonella* (log cfu g^{-1}) in Lettuce, Cabbage, Coriander and Mint collected from various locations of District Swat.

Salmonella

Information regarding *salmonella* the statistical analysis shows a significant change among all the spots (Fig. 4 or Table 4). Results marks that the maximum number of *salmonella* for lettuce (4.05 log cfu g^{-1}), cabbage (4.03 log cfu g^{-1}), coriander (4.04 log cfu g^{-1}) and mint (4.02 log cfu g^{-1}) followed by Kabal region lettuce (3.89 log cfu g^{-1}), cabbage (3.84 log cfu g^{-1}), coriander (3.87 log cfu g^{-1}) and mint (3.83 log cfu g^{-1}) while minimum values for lettuce (3.60 log cfu g^{-1}), cabbage (3.55 log cfu g^{-1}), coriander (3.57 log cfu g^{-1}) and mint (3.51 log cfu g^{-1}) was recorded for the vegetables samples collected from Matta spots. Fresh fruit and vegetables are defenseless to food borne pathogens contamination, at many occasions during the pre and post-harvest handling through waste water irrigation, contaminated soil, insect, animals and human involvement (Beuchat, 2002; Hoyle, 2008 and Hosein *et al*., 2008). According to our study observation the high number of *salmonella* was identified in the vegetables plant samples collected from Mingora region it is because of weather condition and the people used waste water and other waste sludge in the field. Ibenyassine *et al*., (2016) and Steele *et al*., (2005) reported that both human and animals waste disposal may be a major source of pathogenic microorganisms that later contaminate fruits and vegetables in the

fields. Maffei *et al*., (2016) who stated that relative high temperature is very feasible for microorganism's proliferation.

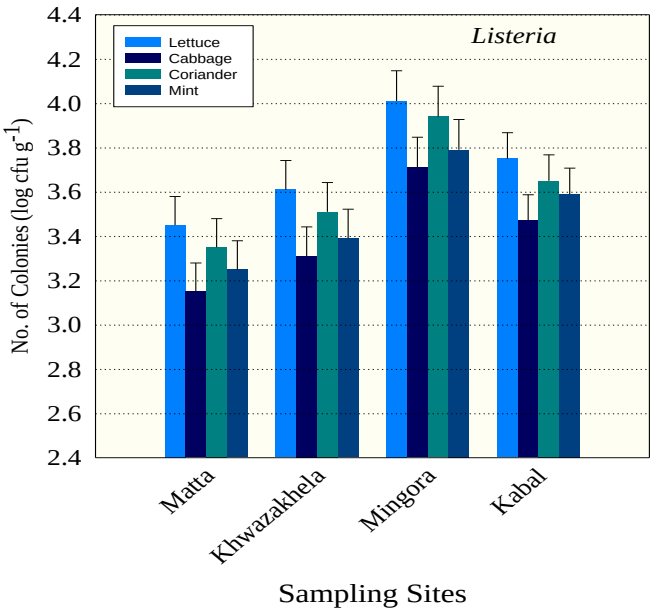

Figure. 5: *Listeria* (log cfu g^{-1}) in Lettuce, Cabbage, Coriander and Mint collected from various locations of district Swat.

Listeria

Statistical analysis of the data shows a significant difference among all the location (Fig. 5 or Table 5). Data regarding for number of *listeria* the more values for lettuce (4.01 log cfu g^{-1}), cabbage (3.71 log cfu g^{-1}), coriander (3.94 log cfu g^{-1}) and mint (3.79 log cfu g^{-1}) was recorded for the vegetables samples collected from Mingora region followed by Kabal location lettuce (3.75 log cfu g^{-1}), cabbage (3.47 log cfu g^{-1}), coriander (3.65 log cfu g^{-1}) and mint (3.59 log cfu g^{-1}) while minimum values for lettuce (3.45 log cfu g^{-1}), cabbage (3.15 log cfu g^{-1}), coriander (3.35 log cfu g^{-1}) and mint (3.25 log cfu g^{-1}) was recorded for the vegetables samples collected from Matta locations. The risk of diseases transmission from pathogenic microorganisms is influenced by the level of contamination, the persistence of pathogen in soil, water and the route of exposure to the crops (Marina and Joseph 2004). Comparing the observation of our findings the maximum number of *listeria* was noted in the vegetable's samples collected from Mingora region it may be due to Mingora is warmer and contaminated area among the other locations. Farmer use waste slush and contaminated water which is a medium for occurrence and proliferation of microbes. Solomon *et al*., (2010) stated that those crops which are grown on the soil having manure fertilizer, wastewater

showed the presence of maximum food borne pathogenic bacteria. Hosein *et al*., (2008) reported that fresh fruit and vegetables are vulnerable to contamination by food borne pathogen.

Table. 1: Total bacterial count (log cfu g^{-1}) in Lettuce, Cabbage, Coriander and Mint collected from various locations of district Swat.

Locations(L)	Vegetables (V)			
	Lettuce	Cabbage	Coriander	Mint
Matta	4.70d	4.66d	4.61d	4.61d
Khwazakhela	4.99c	4.95c	4.92c	4.87c
Mingora	5.58a	5.56a	5.52a	5.49a
Kabal	5.23b	5.19b	5.15b	5.12b
LSD (≥ 0.05)	0.062	0.012	0.053	0.011

Table. 2: Enterobacteriaceae (log cfu g^{-1}) in Lettuce, Cabbage, Coriander and Mint collected from various locations of district Swat.

Locations(L)	Vegetables (V)			
	Lettuce	Cabbage	Coriander	Mint
Matta	4.61d	4.56d	4.54d	4.53d
Khwazakhela	4.89c	4.84c	4.81c	4.79c
Mingora	5.49a	5.46a	5.43a	5.41a
Kabal	5.13b	5.09b	5.06b	5.03b
LSD (≥ 0.05)	0.112	0.023	0.079	0.024

Table. 3: *Escherichia coli* (log cfu g^{-1}) in Lettuce, Cabbage, Coriander and Mint collected from various locations of district Swat.

Locations(L)	Vegetables (V)			
	Lettuce	Cabbage	Coriander	Mint
Matta	3.70d	3.51d	3.61d	3.53d
Khwazakhela	3.78c	3.70c	3.75c	3.67c
Mingora	4.08a	4.04a	4.08a	4.03a
Kabal	3.88b	3.85b	3.87b	3.83b
LSD (≥ 0.05)	0.035	0.082	0.014	0.045

Table. 4: Salmonella (log cfu g-1) in Lettuce, Cabbage, Coriander and Mint collected from various locations of district Swat.

Locations(L)	Vegetables (V)			
	Lettuce	Cabbage	Coriander	Mint
Matta	3.60d	3.55d	3.57d	3.51d
Khwazakhela	3.77c	3.71c	3.87c	3.67c
Mingora	4.05a	4.03a	4.04a	4.02a
Kabal	3.89b	3.84b	3.87b	3.83b
LSD (≥ 0.05)	0.037	0.028	0.016	0.041

Table. 5: *Listeria* (log cfu g^{-1}) in Lettuce, Cabbage, Coriander and Mint collected from various locations of district Swat.

Locations(L)	Vegetables (V)			
	Lettuce	Cabbage	Coriander	Mint
Matta	3.45d	3.15d	3.35d	3.25d
Khwazakhela	3.61c	3.31c	3.51c	3.39c
Mingora	4.01a	3.71a	3.94a	3.79a
Kabal	3.75b	3.47b	3.65b	3.59b
LSD (≥ 0.05)	0.076	0.031	0.044	0.037

CONCLUSION

Consumption of leafy vegetables is widespread due to the numerous health benefits they provide. Contamination of these vegetables with microorganisms is a major concern around the world. It has been determined that leafy vegetables in Swat district contain enough contamination to cause serious health problems such as respiratory problems, kidney problems, reproductive issues, skeletal problems, diarrhea, obesity, vomiting, and so on. In comparison to other studied regions, the number of microorganism colonies is high in leafy vegetable samples collected from Mingora, followed by Kabal. To reduce the risk of heavy metals transmission from food to the human body, quality guidelines and strategies are required.

ACKNOWLEGEMENT

I am most grateful to Allah, who is always at my side and bestows his kindness and blessings on me in all facets of my life. We appreciate the help we received from the academics and lab workers at The University of Agriculture Peshawar in Pakistan's Department of Horticulture throughout the course of my research.

AUTHOR CONTRIBUTIONS

My supervisors, Dr. Mehboob Alam, and I conducted the experiment, collected and analyzed the vegetables sample, and wrote the paper.

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