Asia-Pacific Journal of Science and Technology: Volume: 29. Issue: 03. Article ID.: APST-29-03-10. Research Article

APST

Asia-Pacific Journal of Science and Technology

https://www.tci-thaijo.org/index.php/APST/index

Published by the Research and Graduate Studies,
Khon Kaen University, Thailand

Thermotolerant *Escherichia coli* contamination in vegetables from selected urban farms and wet markets in metro Manila, Philippines at the height of COVID-19 pandemic

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Received 2 March 2023
Revised 10 May 2023
Accepted 19 June 2023

Abstract

The fecal indicator bacterium *Escherichia coli* is one of the leading causes of foodborne diseases in the Philippines and its presence has been detected in agricultural crops especially vegetables, posing public health risks. Thus, surveillance and monitoring of locally available fresh produce is warranted to help address food safety issues. This study surveyed the presence of thermotolerant *E. coli* in vegetables in urban farms and wet markets during the peak of the COVID-19 pandemic (February 2021 to March 2022). A total of 419 vegetable samples from three urban farms and four major wet markets in Metro Manila were gathered. Using molecular and culture techniques, *E. coli* was detected in 13.60% of all the samples obtained. There was a significantly higher percentage of *E. coli* contamination from vegetable samples obtained from urban farms compared to samples obtained from wet markets. However, there was not enough evidence in this study to conclude that season (wet and dry); climatic variables such as average temperature, average rainfall, and average relative humidity; and physicochemical parameters of irrigation water (pH, temperature, salinity, turbidity, and dissolved oxygen) are correlated with *E. coli* contamination in fresh produce. This indicates that there might be other factors that directly impact the level of contamination in vegetables. Measures to improve surveillance and monitoring of contamination must be implemented to ensure proper risk assessment of *E. coli* contamination in agricultural settings and to prevent foodborne diseases.

Keywords: Agricultural crops, COVID-19, *Escherichia coli*, Food safety, Philippines

1. Introduction

Fecal indicator bacteria (FIB), usually referred to as fecal coliforms (FC), are the most common microbiological contaminants of aquatic and freshwater ecosystems. FC are natural inhabitants of the digestive tracts of warm-blooded animals such as cattle, poultry, and humans. These FCs are normally excreted in the feces which are mostly released and washed into streams by surface runoff. Though not all FCs presents are pathogenic, still some species like *Escherichia coli* can be opportunistic pathogens which cause severe illnesses such as gastroenteritis, typhoid fever and dysentery. Because it is not feasible to directly monitor all pathogens, FIB monitoring has been the standard practice which has been one of the measures used in epidemiological studies.

The increased demand in fresh produce in the Philippines causes many challenges for the local fresh produce industry. Microbial contamination is one of the main challenges and a major cause of foodborne diseases globally [1]. While no outbreaks have been reported and documented in the Philippines thus far, it is noteworthy that fresh produce in the Philippines were reported to be contaminated by bacteria, that may potentially cause gastrointestinal infections and pose a risk to public health. For instance, Garcia et al. [1] showed that fresh produce surveyed in selected urban farms in Metro Manila were contaminated by fecal indicators such as the thermotolerant *E. coli* and pathogenic *Salmonella* spp. The same results were obtained by Vital et al. [2] wherein fresh produce sampled in open air markets and supermarkets in major urban and suburban centers in Luzon,

Philippines were found to be contaminated by *E. coli* and *Salmonella* spp., making specific type of vegetables such as lettuce (*Lactuca sativa*) and mung bean (*Vigna radiata*) sprout to be considered as unacceptable or unsatisfactory for human consumption. Altogether, these studies showed that fresh produce in the Philippines are contaminated by bacteria at the production and retail levels.

A variety of factors have been implicated in the microbial contamination of fresh produce, including pre-harvest factors such as raw materials production, and post-harvest factors like storage, transportation, and processing of agricultural products into food products [3]. A major source of microbial contamination of fresh produce is the feces of humans and animals. Fecal contamination of fresh produce may occur through different means. Firstly, fecal contamination may occur through runoffs originating from residential, industrial, and agricultural settings that may be introduced to water and soil in the primary production environment [3]. Particularly, surface runoffs and sewage overflow, especially during the rainy season, from intensive livestock operations (cattle, poultry, and swine) in agricultural farms are considered as a major source of fecal contamination of fresh produce [4]. Fecal contamination may also occur through the direct and open deposition of feces of grazing livestock animals and unleashed stray animals such as cats and dogs.

Recently, numerous community quarantines leading to lockdowns were implemented in the Philippines due to the COVID-19 pandemic. During the COVID-19 crisis, the agriculture sector is one of the industries that were severely affected with an estimated loss of USD 1.47 million from unsold produce alone [5]. Although urban agriculture has already been in practice in Metro Manila long before the pandemic, this period has highlighted its vital role in providing additional food source for the urban dwellers especially at times of crisis wherein strict travel restrictions were implemented. In Quezon City, the local government encouraged its residents to pursue urban gardening by exempting the land used for urban gardens from paying Idle Land Tax. This project resulted in the emergence of many urban gardens in the city thus, providing livelihood among its constituents. The produce from these gardens were also being sold in their respective communities as well. Therefore, it is important to assess the risks and safety of urban agriculture considering its location and the surrounding human activities around it.

This study aimed to assess the incidence of thermotolerant *E. coli* in locally available vegetables produced from urban farms as well as sold in major wet markets in Metro Manila and to determine the effects of season as well as physicochemical parameters of irrigation water to the level of *E. coli* contamination. The information that was gathered in this research can be a baseline for determining the risk associated with contamination on agricultural resources and establishing sufficient data to conduct regulation and policies regarding the elimination and control of these microbial pathogens in agricultural resources. In the Philippines, the Department of Agriculture-Bureau of Agriculture and Fisheries Standards is currently developing microbiological criteria as Philippine National Standards in agricultural products such as vegetables. The data will be of great help in the creation of such microbiological criteria.

2. Materials and methods

2.1 Sampling sites and sample collection

Due to the onset of COVID-19 pandemic, numerous lockdowns took place and there were heavy restrictions regarding access to the laboratories, as well as, in travel and commuting. The sampling sites were urban farms located in Metro Manila per the recommendation of the funding agency as local governments encouraged communities to establish urban gardens. Major wet markets in Metro Manila were also added to widen the sampling coverage and to get a complete picture of the level of contamination. Three urban farms and four wet markets were identified as sampling sites for this study. Random species of vegetables were collected, depending on the availability on the sampling sites. For urban garden sampling, three vegetable samples were obtained from three spots in a plot, and these were combined to be considered as one sample only. For wet market sampling, the following vegetables were selected as target samples: cabbage, carrot, cucumber, sweet potato leaves, water spinach, lettuce, snow cabbage, spring onion, and tomato. Then, five stalls per market from where the target vegetables were obtained were randomly selected. Some vegetables were not available in all the stalls leading to the different sample sizes for each wet market sampling.

For water sampling, surface-level grab water samples (1 L) were stored in sterile containers, placed on ice for transfer, and analyzed within 3 h of sampling. Physicochemical parameters (including water temperature, salinity, turbidity, pH, and dissolved oxygen) were measured using LAQUA WQ-330 (Horiba, Kyoto, Japan). Most of the water samples collected in the urban gardens were either drum water or deep-well water.

2.2 Quantification of fecal indicator organism (E. coli)

Wash solutions of the samples were prepared by adding 30 mL of 0.1% buffered peptone water (BD, Germany) to 25 grams of shredded vegetable samples and shaking vigorously for 30 sec. Wash solution was then subjected

to 10-fold serial dilution. The last two dilutions (10^{-4} and 10^{-5}) were filtered separately using 0.45 um membrane filters and plated on modified mTEC agar (Millipore, USA). Plates were first incubated at 37°C for 2 h and then incubated at 44.5°C for 22 h. Deep violet colonies were considered presumptive *E. coli*. At least three isolated colonies were streak plated on Eosin Methylene Blue (EMB) (BD, Germany) agar plates, and incubated at 37°C for 24 h. Colonies with green to black colonies with or without the presence of green metallic sheen were considered thermotolerant *E. coli*. Samples were re-tested in case the batch per sampling site turned out to be all negative in EMB agar, wherein the preserved wash solutions were used to re-test samples that were negative in EMB agar. Samples that were negative in EMB agar after re-testing were considered negative. Presumptive thermotolerant *E. coli* colonies were transferred to trypticase soy broth (TSB) (BD, Germany) and incubated at 37°C for 24 h. Overnight cultures in TSB were subjected to DNA extraction for use in confirmation of *E. coli*.

2.3 Molecular analysis to confirm identity of E. coli

Confirmation for presence of *E. coli* was done by detection of *uidA* gene by polymerase chain reaction. DNA extraction was done using commercially available DNA extraction kit (G-spin Genomic DNA extraction kit, iNtRON Bio, South Korea) following the manufacturer's instructions. The resulting extracts were subjected to polymerase chain reaction using the MiniAmp Plus (ThermoFisher, USA) thermocycler to amplify the *uidA* gene for *E. coli* confirmation. The primers used to amplify 75-bp *uidA* gene were adapted from Takahashi et al. [2], specifically ECN 1254 F (GCAAGGTGCACGGGAATATT) and ECN 1328 R (CAGGTGATCGGACGCGT). The PCR mix contain 12.5 uL of GoTaq Green Mastermix (Promega, USA), 1 uL each of 1 mM of forward and reverse primers (Oligo, Macrogen, South Korea), 1 uL of DNA template, and 4.5 uL of nuclease free water, resulting to a total of 20 uL reaction Mix. The PCR conditions were as follows: Initial denaturation for 98°C for 2 min, followed by 35 cycles of 95°C for 30 sec, 63°C for 1 min, and 72°C for 1 min. Lastly, a final extension of 72°C for 1 min was done. The positive control used for each run was *E. coli* ATCC 25922 (American Type Culture Collection). The resulting amplicons were visualized using agarose gel electrophoresis.

2.4 Correlation of season and physicochemical parameters to E. coli contamination in produce

The mean CFU concentrations across seasons were compared using the Mann-Whitney U test, while the correlation of climatological parameters (relative humidity (RH), temperature, and rainfall) were analyzed using the Spearman Rank Test. Additionally, the physicochemical parameters of irrigation water and its relation to *E. coli* contamination were analyzed using the Wilcoxon Rank Sum Test and Spearman Correlation test. All tests were performed assuming 95% confidence level. All statistical analyses were performed using Microsoft Excel 2013 and RStudio (2022.07.1 Build 554).

3. Results

3.1 Prevalence of E. coli in vegetables

All *E. coli* colony forming units (CFU) per gram of vegetables were transformed to $\log_{10}$ values for the statistical analyses. Vegetable samples from three urban farms and four major wet markets in Metro Manila were gathered during the peak of the COVID-19 pandemic (February 2021 to March 2022). The presence of thermotolerant *E. coli* was assessed by membrane filtration using mTEC agar. *E. coli* was confirmed by the detection of uidA gene using PCR. Overall, the prevalence of thermotolerant *E. coli* was 13.60% (N = 419) (Table 1). The prevalence was higher in samples obtained from urban gardens (21.13%) compared to the wet market samples (11.78%). Mean CFU of *E. coli* in urban garden samples was significantly higher than that of wet market samples ($p < 0.05$, unpaired T-test).

Table 1 Prevalence, mean colony forming units per gram (CFU/g) of *E. coli* in vegetable samples from urban farms and wet markets in Metro Manila.

Sampling site category	*E. coli* prevalence	Mean $\log_{10}$ CFU/g	Standard error
Wet market (n= 348)	11.78%	3.63	0.14
Urban garden (n= 71)	21.13%	4.47	0.21
Total (n=419)	13.60%	3.85	0.13

Table 2 summarizes the prevalence of *E. coli* by the type and source of vegetable. High *E. coli* CFU was noted particularly in sweet potato (*kamote*) leaves coming from urban garden (5.54 CFU $\log_{10}$) while the lowest was noted in cucumber coming from wet market (3.16 CFU $\log_{10}$). On the other hand, *E. coli* was not detected among the following samples: bitter gourd leaves, arugula, and mustard.

Table 2 Prevalence of *E. coli* by the type and source of vegetables (n=419).

Produce	Source	Prevalence	Mean *E. coli* CFU log_{10}
Amplaya (Bitter Gourd) leaves	Urban Garden (n=3)	0 (0%)	-
Arugula	Urban Garden (n=3)	0 (0%)	-
Cabbage	Wet Market (n=38)	2 (5.26%)	3.59
Carrot	Wet Market (n=42)	3 (7.14%)	4.01
Cucumber	Wet Market (n=42)	6 (14.28%)	3.16
Taro	Urban Garden (n=10)	4 (40%)	3.66
Sweet Potato Leaves	Urban Garden (n=10)	2 (20%)	5.54
	Wet Market (n=32)	2 (12.5%)	3.29
	Total (n=42)	4 (9.52%)	
Water Spinach	Urban Garden (n=22)	8 (36.36%)	4.65
	Wet Market (n=39)	3 (7.69)	3.29
	Total (n=61)	11 (18.03%)	-
Lettuce	Urban Garden (n=6)	0 (0%)	-
	Wet Market (n=37)	6 (16.21%)	3.74
	Total (n=43)	6 (13.95%)	-
Mustard	Urban Garden (n=3)	0 (0%)	-
Spring Onion	Wet Market (n=41)	5 (12.19%)	3.40
Snow Cabbage	Urban Garden (n=11)	0 (0%)	-
	Wet Market (n=37)	7 (18.91%)	3.92
	Total (n=48)	7 (14.58%)	-
Talinum	Urban Garden (n=3)	1 (33.33%)	4.15
Tomato	Wet Market (n=40)	5 (12.5%)	3.80
Total	Urban Garden (n=71)	15 (21.13%)	4.47
	Wet Market (n=348)	42 (11.78%)	3.63
	Total (n=419)	57 (13.6%)	3.85

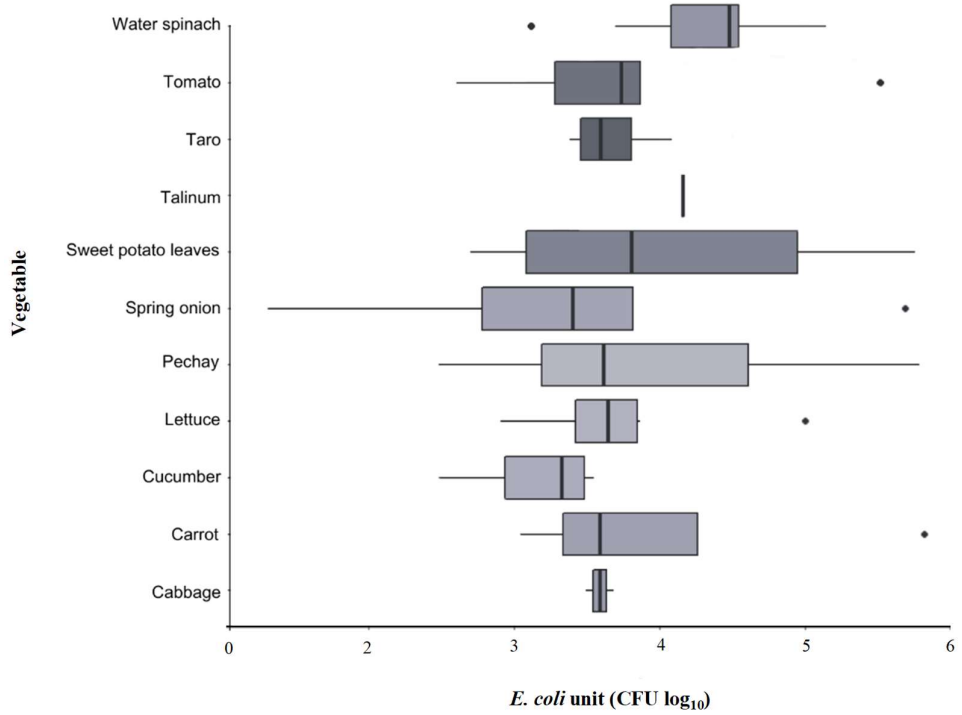

Figure 1 Levels of *E. coli* contamination per type of vegetables.

3.2 Seasonal variation in E. coli contamination in Vegetables

Sampling was done twice in each sampling site-one for the dry season (February - June), and another for the wet season (October - December). For the wet season, 16.43% (N = 207) of the samples obtained were positive for *E. coli*. For the dry season on the other hand, only 10.85% (N = 212) of the samples obtained were positive

for *E. coli.* Table 3 shows the mean and median colony forming units (CFU $\log_{10}$) of *E. coli* per season. Kruskall-Wallis rank sum test showed no significant difference in *E. coli* contamination between wet season and dry season (a = 0.05).

Table 3 *E. coli* contamination comparison between wet and dry season.

Season	Prevalence	Average	Median
Dry season	10.85%	4.04	3.74
Wet season	16.43%	3.73	3.65

To further test the impact of weather to *E. coli* contamination, climatic data (average rainfall, relative humidity, and temperature) seven days prior to urban farm sampling dates were obtained from the Philippine Atmospheric, Geophysical and Astronomical Services Administration (PAGASA). The climatic data for the sampling of wet market was excluded from the analysis since the harvesting dates and production sites were unknown. The Spearman Rank Test between the mean CFU/g log 10 of *E. coli* contamination in Urban Garden and Average Rainfall (mm), RH (%), and Temperature (°C) showed that there was no correlation between these climatic parameters and level of *E. coli* contamination (Table 4). Climatic data for sampling date on urban garden site 3 was not included in the correlation analysis since *E. coli* was not detected in urban garden site 3. Also, climatic data for urban garden site 3 was not yet available from PAGASA as of date of writing.

Table 4 Average rainfall, temperature, and relative humidity collected from the PAGASA Science Garden Satellite, Quezon City, seven days prior to the sampling date.

Climatic factor	Wet season			Dry season	
	Site 1	Site 2	Site 3	Site 1	Site 2
Rainfall	5.38	4.26	0.70	0.06	0.96
Temperature	28.17	27.29	28.30	25.94	25.66
Relative Humidity	81.43	83.57	77.00	70.29	69.86

3.3 Relationship between physicochemical parameters of irrigation water and E. coli contamination

Descriptive statistics using Microsoft Excel 2016 were applied to the data for physicochemical properties of irrigation water from urban gardens. Since the values of salinity and DO results for two irrigation water samples were below detection limit, values were transformed into half of the detection limit for statistical analysis [6]. Figure 2 shows the relationship of the physicochemical quality of irrigation water used in the urban farming sites to the level of *E. coli* colony forming units expressed in $\log_{10}$ (CFU $\log_{10}$). pH, salinity, and dissolved oxygen ranges fell within the acceptable values according to irrigation water guidelines in the Philippines [7]. On the other hand, 10 out of 17 samples have temperatures falling below the recommended temperature for irrigation water according to DENR. This is mainly due to the mode of storage used by the urban farmers (i.e., storing water in drums and use of well water for irrigation). Likewise, high turbidity values were obtained which are mainly due to the modes of storage employed by the farmers. The farmers were noted to use well water, rainwater, and pond water for irrigation and mainly store them in drums that were not properly maintained. This resulted in the poor water quality obtained from the sampling sites. Mann-Whitney test showed no significant difference between the physicochemical parameters of *E. coli*-positive irrigation water and *E. coli*-negative irrigation water samples. Spearman correlation and Kendall's rank correlation Tau showed no evidence of correlation between the average *E. coli* CFU per sampling site and temperature, pH, salinity, turbidity, and DO.

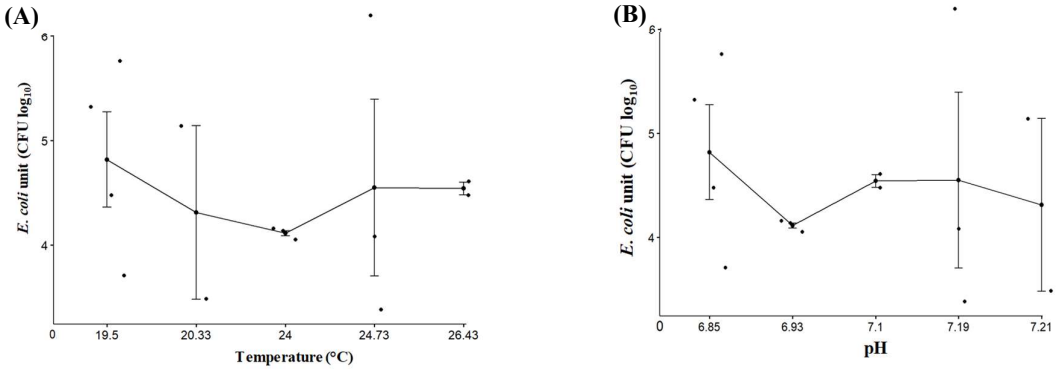

Figure 2 Mean *E. coli* CFU $\log_{10}$ with respect to physicochemical parameters of irrigation water; (A) Temperature, and (B) pH and its error limits using standard error.

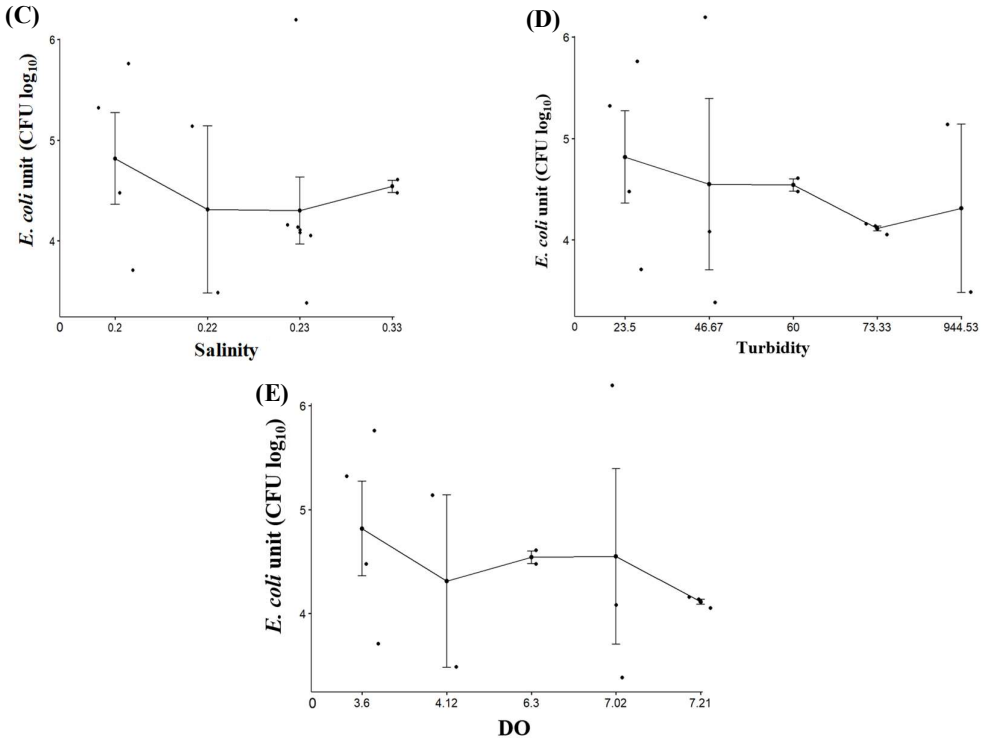

Figure 2 (continued) Mean *E. coli* CFU $\log_{10}$ with respect to physicochemical parameters of irrigation water; (C) Salinity, (D) Turbidity, and (E) DO and its error limits using standard error.

4. Discussion

Raw vegetables are usually incorporated into fresh dishes such as salads and sauces. Consumption of these vegetables is important to one's diet, however, it could lead to several foodborne outbreaks. Bacterial contamination occurs under the influence of various factors such as the presence of roaming and domesticated animals, use of contaminated water, and the lack of food safety measures during harvest, transport, process, and distribution [8]. Bacterial contamination of these vegetables may occur in different stages from cultivation to harvest. Upon contact with contaminated material such as soil or water, bacteria may persist in plant surface, and may also be internalized in plant tissues through natural plant openings such as the stomata or damaged tissues [9]. *E. coli* is one of the most common pathogens causing foodborne outbreaks. *E. coli*, particularly the pathogenic strains (i.e., EHEC, EPEC, STEC), may cause diseases such as diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome among others. However, it is important to note that not all *E. coli* strains are pathogenic, as some *E. coli* strains are part of the normal gut microbiota [10]. This study confirmed the presence of thermotolerant *E. coli* in vegetables, but the identification of pathogenic and non-pathogenic strains was outside the scope of this study. Nonetheless, although the pathogenicity of the *E. coli* isolates is yet to be confirmed, its presence is an indication of fecal contamination in fresh produce [8].

The numerous community quarantines, lockdowns, and travel restrictions during the pandemic resulted in food insecurities in some communities [6]. This crisis led to the recognition of the significance of local food sustainability, hence, the emergence of urban farming in the Philippines to address food security issues in some communities [6]. In light of this, urban farms were chosen as sampling sites for this study. Major wet markets in Metro Manila were also chosen as additional sampling sites for a more comprehensive survey of *E. coli* contamination in vegetables.

According to the data, there is a higher prevalence of *E. coli* in urban gardens as compared to the wet markets. This might be due to the lack of post-harvest processing as compared to the samples coming from wet markets. The usage of rainwater, pond water, as well as well water were noted in the urban farm sampling sites. This practice mainly served economic purposes to the farmers; however, this adds risks of bacterial contamination. Another reason might be cross-contamination by an infected food handler [6]. Furthermore, some domestic animals (cats, dogs, and chickens) were found to be roaming around the urban farms which adds more risk of fecal contamination in both the irrigation water and the soil where the vegetables were planted. Soil could be a source of contamination since it can have a direct contact to the produce which can easily transfer the bacteria [9]. Another possible source of contamination can be irrigation water since it is directly applied to the vegetables. In

this study, the presence of *E. coli* was highest in taro (gabi plant). In contrast, no *E. coli* was detected in bitter gourd (*ampalaya*) leaves, arugula, and mustard (*mustasa*). Figure 1 summarizes the level of *E. coli* contamination per type of vegetables.

Based on the data and statistical analysis performed in this study, it was found that there was no significant difference in the level of *E. coli* contamination between wet and dry season. Contrarily, results obtained from previous studies conducted in various countries showed that wet season produced higher occurrences of contamination [7,11]. According to PAGASA, in the Philippines, the months of June to November are regarded as the wet season while the months of December to May are the dry season. Theoretically, the significant difference in the level of contamination between seasons is attributed to the higher amount of rainfall in the wet season wherein the heavy precipitation leads to increased surface runoff which may possibly be a vehicle for contaminants to spread in soils and water sources [12]. Other studies have observed a positive correlation between contamination and amount of rainfall [13,14], however, in this study, no correlation was found between rainfall and *E. coli* contamination. This may be because the data collected were limited to one satellite site, which is the Science Garden located in Quezon City, since this site is the closest to all the sampling sites from which the fresh produce samples were obtained. Aside from this, other factors such as post-harvest practices, transportation, and storage can be considered as other sources of contamination. It must also be noted that the urban gardens included in this study were noted to be surrounded with residential houses, thus, in the occurrence of rain, household waste runoff, along with wastes from domestic animals that were roaming around the area, may be transported into the urban garden as well as the water used for irrigation.

Relative humidity (RH) and temperature were also analyzed for its correlation to the level of *E. coli* contamination. These two parameters are considered as the most used parameters in determining the optimal conditions for microbial growth. In this study, no correlation was found for both RH and temperature with respect to *E. coli* contamination. The Philippines is a humid country, meaning, RH is expected to be high at around 85%. *E. coli* was found to survive at 40-80% RH [13]. In this study, the RH collected seven days prior to the sampling date fell within the optimum growth range for *E. coli* which might indicate that RH was one of the environmental factors that contributed to the persistence of *E. coli* in the samples from this study, albeit no correlation was observed.

In addition to climatic conditions, another factor that could affect the microbiological integrity of fresh produce is irrigation water quality. This includes the physicochemical parameters of irrigation water such as pH, temperature, salinity, turbidity, and DO. Bacteria are known to grow in an extensive range of temperatures. This is possible since they can adapt their cytoplasmic membrane's fatty acid structure ensuring optimal solute transport and other functions of the membrane [15]. *E. coli* is a bacterium from the family *Enterobacteriaceae* that grows optimally at a temperature of 37°C. It was previously reported that they could grow as high as 53°C and as low as 4°C although these extreme temperatures are not recommended since it reduces the viability of the bacteria [16]. In this study, we observed that the irrigation waters used were within the average (24.06°C) and mean temperature (24.30°C) wherein *E. coli* can survive. However, it was found that it was not directly correlated with the level of *E. coli* contamination in the fresh produce and there was also no significant difference between the temperatures of *E. coli* positive irrigation water compared to the *E. coli* negative irrigation water. This implies that other environmental parameters apart from temperature itself increases the risk of *E. coli* contamination.

The hydrogenionic potential or more so known as pH is the measure of acidity or alkalinity of a solution. It is one of the main parameters used in assessing the suitability of water for agricultural purposes and has direct implications in the microbial quality of soil [17]. In Philippine guidelines, the recommended pH for irrigation of crops is between 6.5-9. The pH of the water samples used in the urban farms are within this acceptable range. In this study, there was no correlation seen between pH of irrigation water and the CFU levels of *E. coli* in vegetables. There was also no significant difference between the pH levels of *E. coli*-positive irrigation water samples compared with *E. coli*-negative samples. This implies that other factors might have contributed to the presence of *E. coli* in the vegetable samples. Many studies have demonstrated the different pH ranges optimal for bacterial pathogens as well as survival of these pathogens in non-optimal pH ranges [18]. *E. coli* is very flexible in terms of the pH ranges where it could persist. *E. coli* can tolerate a high acid environment during gastrointestinal colonization as well as alkaline conditions at the pancreatic ducts [19,20].

Electrical conductivity (EC) pertains to the quantity of ions (dissolved salts) in a solution. An increase in EC due to increased salinities results in a tendency for ion transport interference as well as enzyme activity inhibition which negatively impacts survival of pathogens in soil or water [21]. This negative impact of salinity to *E. coli* growth has been documented in various studies [22,23]. However, in this study, there was no correlation seen between the salinity of irrigation water and the CFU levels of *E. coli* in vegetables. There was also no significant difference between the salinity of *E. coli*-positive irrigation water samples compared with *E. coli*-negative samples. Although a recent study also showed that salinity had no significant effect on the growth of *E. coli* [21], the indistinct relation observed might be due to the short salinity range observed (0.05-0.40 ppt) and the low prevalence of *E. coli* (21.13%, N = 71).

The presence of suspended solids and various microorganisms in water results in reduced water clarity [22]. In this study, there was no correlation found between the high levels of turbidity in the water samples and the level of *E. coli* contamination. One probable reason is that turbidity is not only caused by the presence of pathogenic microorganisms but also by different turbidity-causing materials (TCM) such as natural organic matter, inorganic particles, and other biological particles [23]. In one study, it has been demonstrated that turbidity is correlated with the presence of heterotrophic bacteria [24]. However, it is important to note that these heterotrophic bacteria may compete with pathogenic bacteria such as *E. coli* for nutrient availability [25]. In another study, it was demonstrated that the growth of *E. coli* O157 was considerably restricted in the presence of competitors [26]. This may possibly explain the lack of correlation between the turbidity level and *E. coli* contamination level in this study.

Generally, toxic conditions are perilous to bacteria as exposure to elevated oxygen levels encourages leakage of reactive oxygen species (ROS) which are toxic for cells. The inhibitory properties of molecular oxygen to *E. coli* have been demonstrated in various studies [27]. However, in this study, there was no relationship observed between the levels of DO and levels of *E. coli* contamination. On the other hand, a recent study showed that DO alone did not result in *E. coli* inactivation [28]. The variability of *E. coli's* reaction to DO exposure may be partly explained by various defense mechanisms such as the activation of genes responsible for expression of ROS scavenging elements during exposure to DO-enriched conditions [29]. ROS scavenging systems help protect bacteria from damage resulting from the toxic effects of oxygen exposure.

Overall, the non-correlation of physicochemical parameters of irrigation water, season, and other climatic variables indicates that there might be other environmental factors, biotic factors, and agricultural practices that affect the levels of *E. coli* contamination in vegetable samples. Since COVID-19 pandemic greatly impacted food supply chains leading to food security issues [30], factors that affect contamination in urban agricultural settings need to be continuously monitored to encourage local food sustainability even in the face of a crisis such as COVID-19 pandemic.

5. Conclusion

Thermotolerant *E. coli* was detected in vegetable from urban farms and major wet markets in Metro Manila. *E. coli* contamination was significantly higher from vegetable samples obtained from urban farms compared to samples obtained from wet markets. However, there was not enough evidence in this study to conclude that season and physicochemical parameters of irrigation water are correlated with *E. coli* contamination indicating that there might be other factors in play. Measures to improve surveillance and monitoring of contamination must be implemented to ensure proper risk assessment of *E. coli* contamination in agricultural settings and to prevent foodborne diseases.

6. Acknowledgements

The authors acknowledge the Department of Agriculture - Bureau of Agricultural Research (DA-BAR) of the Philippine Government for the funding support. The authors would also like to express gratitude to Dr. Joseph Ryan G. Lansangan of the School of Statistics, University of the Philippines Diliman for his contribution to the statistical analysis of the study.

7. References

[1] Garcia BC, Dimasupil MA, Vital PG, Widmer KW, Rivera WL. Fecal contamination in irrigation water and microbial quality of vegetable primary production in urban farms of metro Manila, Philippines. J Environ Sci Health B. 2015;50(10):734-43.

[2] Vital PG, Rivera WL, Abello JJ, Francisco JC. Microbiological assessment of fresh, minimally processed vegetables from open air markets and supermarkets in Luzon, Philippines, for food safety. Environ Dev Sustain. 2019;(21):51-60.

[3] Wos A. Interaction between pre-harvest and post-harvest systems and their Implications for socio-economic development. Food Nutr Bull. 1985;7(2):1-6.

[4] Mishra A, Pang H, Buchanan RL, Schaffner DW, Pradhan AK. A system model for understanding the role of animal feces as a route of contamination of leafy greens before harvest. Appl Environ Microbiol. 2017;83(2):e02775-e027716.

[5] United Nations Philippines. Policy Brief Series, Urban food systems and the pandemic August 2021 policy brief series assessing the impact of COVID-19 on food systems and adaptive measures practiced in metro Manila [Internet]. 2021 [cited 2022 November 12] Available from https://philippines.un .org/sites/default/files/2021-08/Urban%20Food%20Systems%20and%20the%20Pandemic.pdf.

[6] Erokhin V, Gao T. Impacts of COVID-19 on trade and economic aspects of food security: evidence from 45 developing countries. Int J Environ Res Public Health. 2020;17(16):5775.

[7] Lipp EK, Kurz R, Vincent R, Palacios CR, Farrah SR, Rose JB. The effects of seasonal variability and weather on microbial fecal pollution and enteric pathogens in a subtropical estuary. Estuaries. 2001;24:266-76.

[8] Deering AJ, Mauer LJ, Pruitt RE. Internalization of *E. coli* O157: H7 and *Salmonella* spp. in plants: a review. Food Res Int. 2012;45(2):567-575.

[9] Nath M, Vandana UK, Choudhury A, Adapa D, Kumar D. Molecular epidemiology and prevalence of *Escherichia coli* contamination in fresh vegetables sold at retails in Silchar, Assam, India. Int J Agri Biol Eng. 2019;35:1-5.

[10] European Centre for Disease Prevention and Control. Facts about Escherichia coli [Internet] 2017. [cited 2023 May 13]. Available from: https://www.ecde.europa.eu/en/escherichiacoliecoli/fact#:~:text=Linked%20In%20Mail-,Escherichia%20coli%20(E.,that%20could%20produce%20serious%20infection.

[11] Kulinkina AV, Mohan VR, Francis MR, Kattula D, Sarkar R, Plummer JD, et al. Seasonality of water quality and diarrheal disease counts in urban and rural settings in south India. Sci Rep. 2016;6(1):20521.

[12] Islam MM, Hofstra N, Islam M. The impact of environmental variables on faecal indicator bacteria in the Betna river basin, Bangladesh. Environ Sci Processes. 2017;4(2):319-332.

[13] Hoeksma P, Aarnink AJ, Ogink NW. Effect of temperature and relative humidity on the survival of airborne bacteria. Wageningen UR Livestock Research; 2015.

[14] Siliakus MF, van der Oost J, Kengen SWM. Adaptations of archaeal and bacterial membranes to variations in temperature, pH and pressure. Extremophiles. 2017;21(4):651-670.

[15] Presser KA, Ratkowsky DA, Ross, T. Modelling the growth rate of *Escherichia coli* as a function of pH and lactic acid concentration. J Appl Environ Microbiol. 1997;63(6):2355-2360.

[16] Kim C, Ndegwa E. Influence of pH and temperature on growth characteristics of leading foodborne pathogens in a laboratory medium and select food beverages. Austin J Nutr Food Sci. 2018;3(1):1031.

[17] Foster JW. *Escherichia coli* acid resistance: tales of an amateur acidophile. Nat Rev Microbiol. 2004;2(11): 898-907.

[18] Evans DF, Pye G, Bramley R, Clark AG, Dyson TJ & Hardcastle JD. Measurement of gastrointestinal pH profiles in normal ambulant human subjects. Gut. 1988;29(8):1035-1041.

[19] Shabala L, Bowman J, Brown J, Ross T, McMeekin T, Shabala S. Ion transport and osmotic adjustment in *Escherichia coli* in response to ionic and non-ionic osmotica. Environ Microbiol Rep. 2009;11(1), 137-148.

[20] Ma J, Ibekwe AM, Crowley DE, Yang CH. Persistence of *Escherichia coli* O157:H7 in major leafy green producing soils. Environ Sci Technol. 2012;46(21):12154-12161.

[21] Jozić S, Morović M, Šolić M, Krstulović N, Ordulj M. Effect of solar radiation, temperature and salinity on the survival of two different strains of *Escherichia coli*. Fresenius Environ Bull. 2014; 23(8):1852-1859.

[22] Grobbelaar, JU. Turbidity. Encyclopedia of Inland Waters. 2009;699-704.

[23] Farrell C, Hassard F, Jefferson B, Leziar T, Nocker A, Jarvis P. Turbidity composition and the relationship with microbial attachment and UV inactivation efficacy. Sci Total Environ. 2018;624:638-647.

[24] Azhdarpoor A, Salehi N, Heidari H, Sarmadipour M, Mahmoudian H. Relationship between turbidity and microbial load of water in Salman Farsi Dam Reservoir. JEMP. 2010;2(2).

[25] Konopka A. What is microbial community ecology? ISME J. 2009;3(11):1223-30.

[26] Vital M, Hammes F, Egli T. Competition of Escherichia coli O157 with a drinking water bacterial community at low nutrient concentrations. Water Res. 2012;46(19):6279-90.

[27] Cheng J, Niu S, Kim Y. Relationship between water quality parameters and the survival of indicator microorganisms–*Escherichia coli*–in a stormwater wetland. Water Sci Technol. 2013;68(7):1650-1656.

[28] Gylienė O, Servienė E, Vepštaitė I, Binkienė R, Baranauskas M, Lukša J. Correlation between the sorption of dissolved oxygen onto chitosan and its antimicrobial activity against *Esherichia coli*. Carbohydr Polyme. 2015;131:218-223.

[29] Shamsudin SN, Rahman MHF, Taib MN, Razak WRWA, Ahmad AH, Zain MM. Analysis between *Escherichia coli* growth and physical parameters in water using Pearson correlation. ICSGRC IEEE. 2016;7:131-136

[30] Knorr D, Khoo CSH. COVID-19 and food: challenges and research needs. Front in Nutr. 2020;7:598913.