Pathogen and pesticide contamination in cabbage grown from, Dalaguete, Cebu, Philippines

Christine Ardelle O. Marquez[1,2] and Kenneth Joseph C. Bureros[1,3,*]

[1]*Department of Biology and Environmental Science, College of Science, University of the Philippines Cebu, Gorordo Avenue, Lahug, Cebu City, Cebu, Philippines 6000*
[2]*Department of Biological Sciences and Biotechnology, Chungbuk National University, Cheongju, Republic of Korea 28644*
[3]*Marine and Pathogenic Microbiology Laboratory, Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung City, Taiwan 80424*
**Correspondence: kcbureros@g-mail.nsysu.edu.tw*

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ABSTRACT

Food safety is one of the long-sought problems in the world. Chemical and biological characteristics must be considered when evaluating the safety of various food products. This study aimed to assess the food safety of cabbages grown from Mantalongon, Dalaguete, Cebu. Cabbage samples acquired from three different farms in Mantalongon were tested for the presence of *Salmonella* spp. (and other potentially pathogenic bacteria) and pesticide (cypermethrin) residues. To detect the presence of *Salmonella* spp., pre-enrichment and enrichment methods were done. For the analysis of cypermethrin residue, samples were subjected to gas chromatography. No putative *Salmonella* spp. colonies (with a black center) were isolated However, other potentially pathogenic bacteria suspected of *Escherichia coli*, *Enterobacter* sp., or *Shigella* sp. were isolated Cypermethrin concentrations (0.006-0.054 mg kg^{-1}) were within the maximum residue limit. Knowing that some vegetables are eaten raw or slightly cooked, consumers are at risk of ingesting residual amounts of pesticides and are prone to bacterial infection. Hence, the food safety of fresh vegetables should be monitored from local farms to markets.

Keywords: *Brassica oleracea* var. *capitata* L., cypermethrin, food safety, gas chromatography, *Salmonella*

INTRODUCTION

One of the major problems worldwide is food insecurity. Food security is achieved by having enough food accessible and safe food for consumption. Because the world is so preoccupied with problems of the former, the latter is usually disregarded, resulting in the lack of adequate data about monitoring and standards. Another challenge regarding food safety actions is the reactive, rather than preemptive government approach towards food safety issues, such as food poisoning incidences and substandard fresh and processed foods in the market (Collado et al. 2015).

Various parameters are considered when assessing the safety of food produce. They include the monitoring of biological and chemical hazard agents in these foods. The biological aspect refers to the pathogens that contaminate food. The chemical element is about the toxic substances that can cause (acute or chronic) toxicity to the consumers (Singh et al. 2019).

Illnesses caused by pathogen contamination in food items have become significant causes of morbidity and mortality worldwide. In the United States, there are 31 primary foodborne pathogens, but the most significant ones are *Salmonella* nontyphoidal, *Campylobacter*, *Listeria*, and Shiga toxin-producing *Escherichia coli* Castellani and Chalmers 1919 (Adley and Ryan 2016). In fact, in the Philippines alone, the Department of Health (DOH) reported 15,524 nationwide cases of pathogen-caused illnesses from 01 January to 03 June 2017 (DOH 2017). In particular, *Salmonella* spp. have grown so much attention due to its generally high prevalence worldwide and its increased resistance to various antibacterial drugs (Threlfall 2002). These pathogens are mainly acquired from eating raw or improperly cooked meat, egg, chicken, and poultry products (Howard et al. 2012; Bodhidatta et al. 2013). However, meat and poultry products are not the only sources of *Salmonella*; fresh vegetables and herbs, including those of the leafy variety, have also been implicated as vehicles for transmitting microbial foodborne disease worldwide (Aycicek et al. 2006; Beuchat 2006). In addition, there has been an increase in the incidence of *Salmonella* spp. contamination in fresh fruits and vegetables (Quiroz-Santiago et al. 2009; Vital et al. 2014).

In Cebu, Philippines, the major vegetable providers are the farms of Mantalongon in the Municipality of Dalaguete. Their primary vegetable produce includes cabbage, "pechay," and spring onions. Among these vegetables, cabbage is one of the staple ingredients in many Filipino recipes. However, it risks bacterial pathogen contamination such as *Salmonella* spp. due to the poor agricultural practices and unguided post-harvest practices (Liu et al. 2013).

Besides bacterial pathogens, another food safety concern in cultivated cabbages in Cebu is pesticide residues. Mantalongon cabbage farmers depend heavily on pesticides application to protect cabbage against the diamondback moth (Lim 2014). Their most active ingredient is cypermethrin, chlorpyrifos, and spinetoram (Lu 2012). Pesticide application leads to higher yields and better-quality products. Yet, its misuse and misapplication significantly affect the health of the farmers, the people living closer to these farms, and consumers of the contaminated food produce and water due to runoff water containing pesticides. Health records of the municipality indicate an increase in mortality among farmers and an increased number of cases of miscarriages (Lu 2005). These occurrences are symptoms of pesticide poisoning.

According to the local authorities in Mantalongon, Dalaguete, there is no food safety program that monitors the quality of farm-produced vegetables. Furthermore, eating fresh and half-cooked leafy vegetables increased risk of food poisoning associated with the consumption of fresh produce with pesticides and pathogenic bacteria.

This study aimed to assess the safety of cabbage (*Brassica oleracea* var. *capitata* L.) heads grown from Mantalongon, Dalaguete, Cebu. Specifically, it (1) determined the presence of *Salmonella* spp. and other bacteria in cabbages, and (2) determined the cypermethrin residue present in cabbages from the mentioned locality.

METHODS

Sample Collection

Cabbage samples were acquired from three different crop-producing farm barangays of the Municipality of Dalaguete, Cebu, Philippines. Sample one was taken from sitio Alang-alang of barangay Mantalongon. Sample 2 from the same barangay but in another site. Sample 3 was from barangay Tabon beside Mantalongon. The acquisition of samples followed a random opportunistic sampling technique, which depended on which farms were producing cabbage crops during the sampling. Cabbage samples were placed in properly labeled clean plastic bags stored in a cooler with ice (0-4°C) before being transported to the laboratory and immediately analyzed within 24 h.

Isolation and Detection of *Salmonella* spp.

Microbial culture. *Salmonella* spp. were detected on cabbage samples using the method by Andrews et al. (2018). Briefly, 25 g of the cabbage samples were aseptically chopped then weighed It was then mixed with 225 ml 0.5% lactose broth (Sigma-Aldrich, USA) and was incubated at 35°C for 24 ± 2 h for pre-enrichment. From the lactose broth culture, an aliquot of 1 ml was transferred into a 10 ml Rappaport-Vassiliadis (RV) medium (HiMedia, India) for *Salmonella* spp. enrichment. The enrichment cultures, done in duplicates, were then incubated at 42°C for 24 ± 2 h. Aliquots of homogenized samples from the RV medium were serially diluted up to 10^{-8} in peptone water, and the last three dilutions (i.e. 10^{-6}, 10^{-7}, and 10^{-8}) were plated on xylose lysine deoxycholate agar (XLDA, HiMedia, India). These steps were also done in duplicates.

Cypermethrin Residue Analysis

Sample preparation. Traces of cypermethrin residue were detected on cabbage samples using GC-ECD analysis (Calinawan et al. 2016). Fifteen grams of homogenized sample was added with 20 ml of 1% acetic acid in acetonitrile and mechanically shaken for 15 min. QuEChERS extraction salts 6 g $MgSO_4$ (Univar, Australia) and 1.5 g NaOAc (Scharlau, Spain) were added to the mixture, then vigorously shaken by hand for a minute, then another minute by vortex mixer. The sample was centrifuged for 10 min then the supernatant was transferred to a beaker. The extract was concentrated to about 5-7 ml at 45˚C in a water bath. The concentrated extract was transferred to a dispersive solid phase extraction tube containing $MgSO_4$, primary-secondary amine, and C18, then vigorously shaken for 1 min and centrifuged for 10 min. In a water bath, the supernatant was decanted to a beaker and evaporated to dryness at 45˚C in a water bath. The extracted residue was redissolved with 2 ml ethyl acetate then analyzed by gas chromatography with an electron capture detector (GC-ECD).

GC-ECD analysis. The extracts were analyzed for the presence of cypermethrin using GC-ECD. The working condition for the GC-ECD analysis is summarized in Table 1.

RESULTS

Pathogens Isolated from Cabbages

Among the three cabbage samples tested, all of the lactose broth pre-enrichment tubes produced gas

(Table 2), and there were no isolated *Salmonella* colonies with a black center. However, other potentially pathogenic species of bacteria were isolated (Table 3). Certain species isolated had opaque yellow colonies with entire margins (Figure 1A) and translucent red to pink, punctiform colonies (Figure 1B).

Cypermethrin Residue in Cabbages

Cypermethrin residue was detected in the samples from barangay Tabon. The other two samples have residue concentrations below the detection limit of 0.006 mg kg^{-1} (Table 4).

Table 1. Gas chromatography with an electron capture detector.

Instrument	Perkin Elmer Clarus 500		
Detector	ECD		
Column	Rtx-CLP, 0.32 μm × 0.32 mm × 30 m		
Oven Temperature	Rate, °C min^{-1}	Initial temperature, °C	Hold, min
	-	230	3
	15	290	5
Carrier gas	Nitrogen, UHP		
Column flow rate	2.5 ml min^{-1}		
Injection volume	1 μl		

Table 2. Growth and gas production of pre-enriched endogenous bacteria from the sampled cabbages. Coliforms such as *E. coli* produce gas in lactose broth tubes while Salmonella does not.

Sample	Sample Source	Growth (Turbidity)	Gas Production
1	Sitio Alang-alang, Mantalongon, Dalaguete, Cebu	✓	✓
2	Sitio Private, Mantalongon, Dalaguete, Cebu	✓	✓
3	Barangay Tabon, Dalaguete, Cebu	✓	✓

Table 3. Number of putative *Salmonella* colonies with black center and other potentially pathogenic bacteria isolated from the three cabbage samples.

Sample	Sample Source	*Salmonella* sp.	Yellow Colonies	Red/Pink Colonies
1	Sitio Alang-alang, Mantalongon, Dalaguete, Cebu	0	✓	✓
2	Sitio Private, Mantalongon, Dalaguete, Cebu	0	✓	✓
3	Barangay Tabon, Dalaguete, Cebu	0	✓	✓

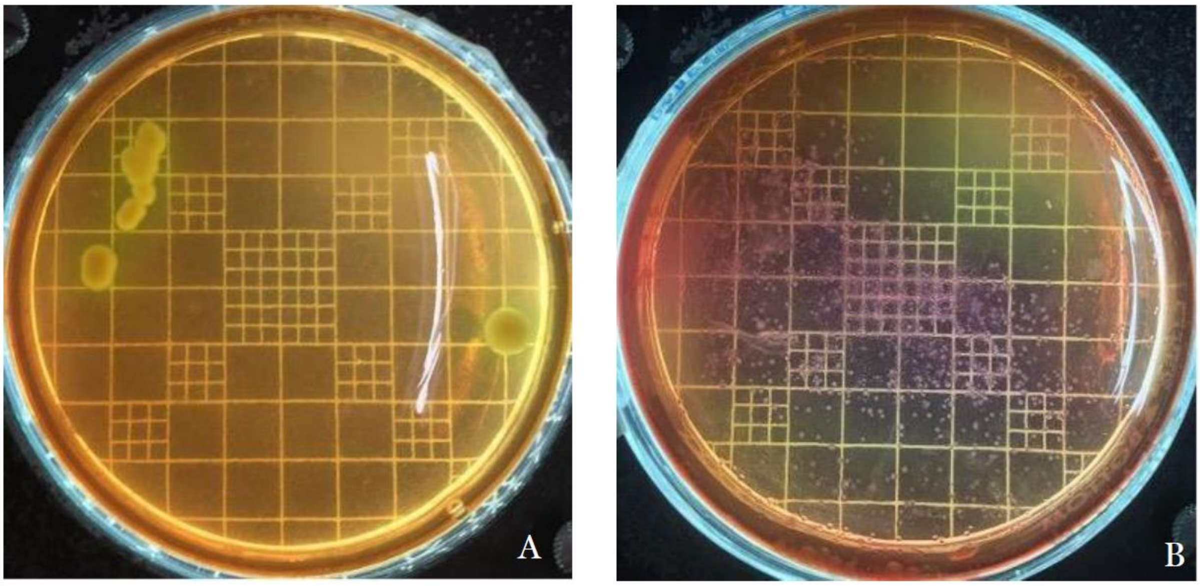

Figure 1. (A) Opaque yellow colonies with entire margin. (B) Translucent red to pink colonies with punctiform shape.

Table 4. Concentration of cypermethrin residue in the three cabbage samples compared to allowable limit based on the studies by Zhang et al. 2007 and Akomea-Frempong et al. 2017.

Sample	Sample Source	α-Cypermethrin, mg kg^{-1}	Allowable Limit, mg kg^{-1}
1	Sitio Alang-alang, Mantalongon, Dalaguete, Cebu	< 0.006	1
2	Sitio Private, Mantalongon, Dalaguete, Cebu	< 0.006	
3	Barangay Tabon, Dalaguete, Cebu	0.054	

DISCUSSION

Presence of Bacteria in Cabbages

Kim et al. (2022) explained that *Salmonella* spp. is a prominent foodborne pathogen that poses a health risk to the general public. *Salmonella* is responsible for over 450 deaths and 1.2 million illnesses each year. Hence, the absence of putative *Salmonella* spp. in the cabbage samples is ideal because the acceptable *Salmonella* spp. content in any food samples is zero (Table 5). The sources of pathogenic bacteria are usually from eggs, dairy products, and meats (Crum-Cianflone 2008). Thus, its absence in leafy vegetables is unusual. Also, Patel and Sharma (2010) reported that the attachment strength of *Salmonella* was significantly lower on cabbage compared to Romain lettuce and other leafy vegetables. Kuan et al. (2017) did not detect *Salmonella* spp. in cabbages from Kuala Lumpur, Selangor, and Putrajaya.

Conversely, Odu and Okomuda (2013) and Saw et al. (2020) found 42.9% and 3.3% of their cabbage samples, respectively. Such differences in detecting foodborne pathogens are common as they arise during pre- and post-harvest handling, especially in hygienic and agricultural practices.

Isolated yellow colonies are suspected to be under the genera of *Enterobacter* and *Escherichia* (HiMedia Laboratories 2015). Some *Enterobacter* spp., like *E. cloacae* and *E. aerogenes* Hormaeche and Edwards, 1960, are normal microflora in the intestinal tracts of humans and animals (Davin-Regli and Pagès 2015). Members of genus *Escherichia*, like *E. coli*, are likewise normal microflora in the guts of humans and animals. The presence of normal intestinal flora in the food samples indicates fecal contamination in the farm. Fecal contamination in the sampled farms is highly possible because many farmers use animal manure as organic fertilizer due to its availability, inexpensiveness, and relative safety instead of chemical fertilizers.

Despite being normal intestinal flora in human guts, bacteria can cause infections due to their opportunistic characteristic. *Escherichia coli* and *Enterobacter* are mainly normal flora of the large and small intestines, respectively. However, they go into the stomach when ingested, damaging the stomach lining, leading to diarrhea and vomiting (Evans and Evans 1996).

Isolated red or pink colonies are suspected of *Shigella* spp. or other *Salmonella* spp. or strains that do not form black-centered colonies (ISO 21567 2004; HiMedia Laboratories 2015). They are ubiquitous pathogens that are common contaminants of raw vegetables. Members of the genus *Shigella* are the leading cause of shigellosis or traveler's diarrhea, wherein it is transmitted through ingestion—usually through fecal-oral contamination from previously infected organisms (Hale and Keusch 1996). They typically thrive in areas where there is poor sanitation. Therefore, if the species isolated are indeed *Shigella* spp., then the bacterial load (in CFU g^{-1}) must be determined to assess whether the sampled farms need improvements in sanitation.

Table 5. Acceptable amount of *Salmonella* spp. in food from various countries.

Acceptable amount of *Salmonella* spp.	Country	Sources
0	China	National Health and Family Planning Commission of People's Republic of China (2013)
0	Ireland	Institute of Medicine and National Research Council (2003)
0	Australia	
0	New Zealand	
0	Canada	

Salmonella spp. that do not form black-centered colonies and only form translucent red colonies are *S. enterica* Le Minor and Popoff 1987. However, these serovars only constitute the minority of the entire genus (ISO 6579 2002), which means that the probability of the isolated species being *Salmonella* is less likely.

The putative identities of the isolated bacteria are *E. coli*, *Enterobacter* sp., or *Shigella* sp. The presence of *E. coli* or *Enterobacter* sp. indicates fecal contamination since both of these bacteria are normal intestinal microflora of many animals. The use of animal manure as organic fertilizer can cause such contamination. On the other hand, *Shigella* spp. are ubiquitous pathogens that commonly contaminate raw vegetables (Hale and Keusch 1996). They thrive in areas with low sanitation; thus, their presence suggests improving the farms' sanitation practices.

Cypermethrin Residue in Cabbages

The persistence of cypermethrin residues in cabbages could be due to several reasons. One of the reasons could be farmers' negligence in withholding time after pesticide application. A pesticide's withholding period is the time that must elapse between its last application and the day of the harvest. Farmers disregard withholding time to meet the high market demands for crops, overcome the unpredictable unseasonal storms, and compensate for a daily source of income. However, farmers must be educated and ensure strict adherence to pesticide withholding time to yield lower or no pesticide residues in cultivated produce from the surveyed area.

Another contributing factor to the persistence of cypermethrin residue is the excessive amounts of pesticides used Calinawan et al. (2017) reported that most farmers in the study sites have no training about the pesticides' recommended doses and spray intervals. They mostly base these decisions on their experience as to which amount they perceive is most effective. They only consulted pesticide dealers for the recommendation of pesticide use, but only a few were found abiding by the advice of agriculture experts. Based on personal communication with the farmers, pesticide contamination of cabbages is expected to happen more frequently over the succeeding years due to excessive use of pesticides.

The detected residue in Sample 3 (0.054 mg kg^{-1}) was within the allowable cypermethrin concentration limit of 1 mg kg^{-1} for cabbages (Zhang et al. 2007; Akomea-Frempong et al. 2017). Such low concentration could be attributed to the current farming practices. Lim (2014) reported that Mantalongon farmers planted odorous flowers that naturally repel cabbage pests. The farmers also planted "trap crops," which are weeds that the diamondback moths prefer over cabbages. Some farmers also developed a natural pesticide made from "tubli" vine (*Derris elliptica* (Wall.) Benth 1860) to kill cabbage insect pests. The presence of wasps on the farm also helps control cabbage insect pests. Calinawan et al. (2016) also detected low cypermethrin residue values in Mantalongon cabbages. They explained that the low concentration of pesticide residue could be due to weather conditions and the frequency of pesticide application. Rainfall can disperse the pesticide residue to other areas, wash out the pesticide from the cabbage leaves, and leach the pesticide from the vegetable to the soil.

Currently, no existing norms for pesticides in the Philippines are established Thus, other international guidelines, such as the Maximum Residue Limit issued by the European Commission (2022), were used for comparison. Even though the cypermethrin levels are within the permissible limit, this study proves that pesticides residues persist in post-harvest cabbage heads. Following previous reports (Lim 2014; Calinawan et al. 2016; Nisha et al. 2021), routine testing of active substances, additives, and pesticide carrier materials could be done in the future to assure food safety. Adopting appropriate agricultural practices and quality control techniques as tactics for producing safe food can help to reduce health risks for consumers. It is also good to conduct extensive pesticide monitoring on environmental samples in the area. This will make implementing an effective risk assessment strategy easier to guide suitable interventions.

In conclusion, cabbages grown from Mantalongon and Tabon, Dalaguete, supplied to most places of Cebu and some neighboring provinces are contaminated with potentially pathogenic bacteria. While the pesticide residue levels in cabbage are within acceptable limits. This observation suggests that food safety assessments must be done regularly to create a more systematic approach in preventing biological and chemical contaminations of fresh produce. In future investigations, it is recommended to collect more samples from other farms in Dalaguete to reflect a larger sample size and reach a more thorough conclusion. A periodic examination of the produce is also recommended (e.g. quarterly in a year) to evaluate the quality of the cabbages produced The local government can initiate regular education and integrated pest management training programs for farmers on proper and safe pesticide use. Training and education on proper hygienic handling, transportation and storage of vegetables can also be extended to develop a hygienic management guide to monitor and assess bacterial pathogen contamination in fresh vegetables from farm to market place and to avoid bacteriological food spoilage and contamination that can lead to related health issues.

Furthermore, biochemical and molecular testing is also recommended to confirm the identity of the bacterial isolates. Confirmation of the identities of the bacterial isolates can help in the decision-making process to determine which aspects of food safety and quality assurance can be improved Lastly, this study is a fundamental guide for future food safety studies on cultivated food produce such as cabbage to set maximum residue limits for biological and chemical hazards in food in the Philippines as food safety standards.

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