Factors Associated with Bacteria Detection in Water in the Coastal Areas, Southern Thailand

Kritsanee Ruangsombat

A Thesis Submitted in Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Marine and Coastal Resources Management
Prince of Songkla University
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Author Miss Kritsanee Ruangsombat

Major Program Marine and Coastal Resources Management

Major Advisor

....
(Dr. Prakrit Noppradit)

Co-advisor

....
(Assoc. Prof. Dr. Apiradee Lim)

Examining Committee :

......................................Chairperson
(Assoc. Prof. Dr. Phattrawan Tongkumchum)

....Committee
(Dr. Prakrit Noppradit)

....Committee
(Assoc. Prof. Dr. Apiradee Lim)

....Committee
(Assoc. Prof. Dr. Siriporn Pradit)

....Committee
(Asst. Prof. Dr. Mathinee Yucharoen)

The Graduate School, Prince of Songkla University, has approved this thesis as fulfillment of the requirements for the Doctor of Philosophy Degree in Marine and Coastal Resources Management

....
(Asst. Prof. Dr. Thakerng Wongsirichot)
Acting Dean of Graduate School

This is to certify that the work here submitted is the result of the candidate's own investigations. Due acknowledgement has been made of any assistance received.

....Signature
(Dr. Prakrit Noppradit)
Major Advisor

....Signature
(Assoc. Prof. Dr. Apiradee Lim)
Co-advisor

....Signature
(Miss Kritsanee Ruangsombat)
Candidate

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....Signature
(Miss Kritsanee Ruangsombat)
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ชื่อวิทยานิพนธ์ ปัจจัยที่มีความสัมพันธ์กับการตรวจพบเชื้อแบคทีเรียในน้ำอุปโภคน้ำบริโภคบริเวณพื้นที่ชายฝั่งทะเลภาคใต้ของประเทศไทย

ผู้เขียน นางสาวกฤษณี เรืองสมบัติ

สาขาวิชา การจัดการทรัพยากรทะเลและชายฝั่ง

ปีการศึกษา 2566

บทคัดย่อ

โรคติดต่อที่มีสาเหตุจากน้ำบริเวณภาคใต้ของประเทศไทยเป็นปัญหาสุขภาพและสาธารณสุขที่สำคัญ สาเหตุหลักเกิดจากอุปโภคบริโภคน้ำปนเปื้อนแบคทีเรียเกินมาตรฐาน และพบการระบาดของโรคติดต่อทางน้ำอย่างต่อเนื่อง การศึกษาปัจจัยที่มีความสัมพันธ์ต่อการปนเปื้อนแบคทีเรียในน้ำอุปโภคบริโภคบริเวณภาคใต้ของประเทศไทยจึงมีความสำคัญและต้องรีบดำเนินการ ข้อมูลคุณภาพน้ำรวบรวมจากรายงานประจำปีกรมวิทยาศาสตร์การแพทย์ระดับภูมิภาค ประกอบด้วย 2 เขตสุขภาพ คือ เขตสุขภาพที่ 11 (ศูนย์วิทยาศาสตร์การแพทย์ที่ 11 สุราษฎร์ธานี ศูนย์วิทยาศาสตร์การแพทย์ที่ 11/1 ภูเก็ต) หรือภาคใต้ตอนบนของประเทศไทย และเขตสุขภาพที่ 12 (ศูนย์วิทยาศาสตร์การแพทย์ที่ 12 สงขลา ศูนย์วิทยาศาสตร์การแพทย์ที่ 12/1 ตรัง) หรือภาคใต้ตอนล่างของประเทศไทย ข้อมูลสิ่งแวดล้อม ได้แก่ ข้อมูลอุตุนิยมวิทยาจากสถิติรายปีสำนักงานสถิติแห่งชาติ เป็นเวลา 21 ปี (พ.ศ. 2545 ถึง พ.ศ. 2565) จำแนกตัวแปรเป็น 2 กลุ่ม คือ 1) ตัวแปรกำหนด ประกอบด้วย เขตสุขภาพ จังหวัดที่ผลิต ปีที่ผลิต ชนิดน้ำ ชนิดแบคทีเรีย อุณหภูมิในอากาศและปริมาณน้ำฝน และ 2) ตัวแปรผลลัพธ์ คือ จำนวนตัวอย่างน้ำที่ตรวจพบแบคทีเรียปนเปื้อนไม่ผ่านมาตรฐาน จากนั้นตรวจสอบความสัมพันธ์ระหว่างตัวแปรด้วยการทดสอบไคสแคว์ และตัวแบบลอจิสติกรีเกรสชั่นพหุคูณทำนายความน่าจะเป็นการตรวจพบแบคทีเรียปนเปื้อนไม่ผ่านมาตรฐาน ประเมินประสิทธิภาพการทำนายของตัวแบบด้วยเส้นโค้งอาร์โอซี ผลการศึกษาพบว่าเขตสุขภาพ และปีที่ผลิต เป็นปัจจัยที่มีผลกระทบต่อการตรวจพบโคลิฟอร์มแบคทีเรียปนเปื้อนไม่ผ่านมาตรฐานในน้ำดื่มบรรจุภาชนะที่ปิดสนิท บริเวณภาคใต้ของประเทศไทย โดยเขตสุขภาพที่ 11 มีโอกาสตรวจพบการปนเปื้อนโคลิฟอร์มแบคทีเรียสูงกว่าเขตสุขภาพที่ 12 ประสิทธิภาพการทำนายของตัวแบบให้ค่าความถูกต้องร้อยละ 63 การศึกษาในภาพรวม พบว่า เขตสุขภาพ ปีที่ผลิต ชนิดของน้ำ ระดับอุณภูมิในอากาศ และระดับปริมาณน้ำฝน คือปัจจัยที่มีอิทธิพลต่อการตรวจพบการปนเปื้อนแบคทีเรีย 4 ชนิดเกินมาตรฐาน โดยทุกปัจจัยมีผลกระทบต่อการตรวจพบ TCB และ *E. coli*,

เขตสุขภาพ และชนิดของน้ำมีผลกระทบต่อ *Salmonella* spp., ในขณะที่มีเฉพาะชนิดของน้ำมีผลกระทบต่อ *S. aureus.* ประสิทธิภาพการทำนายของตัวแบบให้ค่าความถูกต้องร้อยละ 61, 68, 76, and 81 ตามลำดับ โดยเขตสุขภาพที่ 11 พบแบคทีเรียปนเปื้อนสูงกว่าเขตสุขภาพที่ 12. เมื่อศึกษาข้อมูลเชิงพื้นที่ในเขตสุขภาพที่ 11 เป็นรายจังหวัด พบว่า จังหวัดชุมพรมีโอกาสตรวจพบแบคทีเรียเกินมาตรฐานสูงที่สุด ซึ่งตรวจพบไม่ผ่านมาตรฐานมากที่สุดในปี 2565 เมื่อจำแนกรายชนิดของน้ำพบว่าน้ำใช้ในครัวเรือนมีความเสี่ยงปนเปื้อนแบคทีเรียมากที่สุด โคลิฟอร์มคือแบคทีเรียที่ตรวจพบได้มากที่สุด และยังพบว่าปริมาณการปนเปื้อนของแบคทีเรียแปรผันตามปริมาณน้ำฝนที่เพิ่มขึ้น โดยประสิทธิภาพการทำนายของตัวแบบให้ค่าความถูกต้องร้อยละ 82 จากการศึกษาดังกล่าวสรุปได้ว่าปัจจัยด้านสิ่งแวดล้อมและปัจจัยอื่นๆ ที่เกี่ยวข้อง ได้แก่ เขตสุขภาพ จังหวัด ปีที่ผลิต ชนิดของน้ำ ชนิดของแบคทีเรีย ระดับอุณหภูมิในอากาศ และระดับปริมาณน้ำฝนมีอิทธิพลต่อความชุกและการตรวจพบการปนเปื้อนแบคทีเรียไม่ผ่านมาตรฐานบริเวณภาคใต้ของประเทศไทย ดังนั้นเพื่อความปลอดภัยของผู้บริโภค ผู้ผลิตและหน่วยงานที่เกี่ยวข้องต้องตรวจสอบ จัดการ และดำเนินการตามขั้นตอนเพื่อควบคุมคุณภาพน้ำให้สะอาดมีคุณภาพมาตรฐานตามกฎหมายกำหนด ตั้งแต่กระบวนการผลิต การขนส่ง จนส่งมอบน้ำไปยังผู้บริโภค ไม่ให้ได้รับผลกระทบจากสถานที่ผลิต สภาพแวดล้อม หรือการเปลี่ยนแปลงของสภาพอากาศ เช่น อุณหภูมิอากาศ ปริมาณน้ำฝน ฤดูกาล เป็นต้น

คำสำคัญ : ปัจจัย, ความสัมพันธ์, การปนเปื้อนแบคทีเรีย, น้ำอุปโภคบริโภค, ภาคใต้ของประเทศไทย

Thesis Title Factors associated with bacteria detection in water in the coastal areas, Southern Thailand

Author Miss Kritsanee Ruangsombat

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Academic Year 2023

ABSTRACT

Water-borne diseases have been important health and public health issue in southern Thailand. The major reason, that consumption water was polluted with microorganisms that exceed the standard and discovered outbreaks of water-borne disease on an ongoing basis. The investigation of factors affecting bacterial contamination in water was critical and must be completed as urgent. Water quality data were gathered from the Department of Medical Sciences' annual reports at two Public Health Areas (PHA): PHA 11 (RMSC 11, RMSC 11/1) or upper south and PHA 12 (RMSC 12, RMSC 12/1) or lower South. Environmental data such as meteorological (air temperature and rainfall), was collected from yearly statistics, National Statistical Office for 21 years (2002 to 2022). PHA, RMSC, provinces of production, years of production, water types, bacterium types, air temperature, and rainfall were the determinant variables. Bacterial contamination exceeding the standard was the outcome. The association was analyzed by using a chi-square test and used the multiple logistic regression model to predict the probability of detection of bacterial contamination above the standard. The ROC curve was used to evaluate the model's performance. The results from this study showed that the location and year of manufacture were factors influencing TCB contamination higher than standard in sealed container drinking water in Thailand's southern region. PHA 11 was more likely to be contaminated with bacteria than PHA 12. The model's prediction performance was 63% accurate. The overall investigation discovered that PHA, years of manufacture, water types, air temperature, and rainfall were the factors associated with the contamination of four types of bacteria that exceeded the criterion. All those factors were risking the detection of TCB and *E. coli*. PHA and water types impacted *Salmonella* spp., whereas only water types were affecting *S. aureus*. The model's prediction performance was 61, 68, 76, and 81 percent accurate, respectively. PHA 11 found more bacterial contaminants than PHA 12. When reviewing the spatial data by the province in PHA 11, it was found that Chumphon province had the highest probability of bacterial contamination that exceeded the standard. The pollution was maximum in 2022. The most susceptible to bacterial contamination was the consumption water. The most detectable kind of bacteria is TCB. The level of pollution was also discovered to vary with increasing rainfall and the model's prediction performance was 82% accurate. According to the findings of the study, PHA, RMSC, provinces, years of production, water types, bacterium types, air temperature levels, and rainfall levels had influenced the prevalence and bacterial contamination in southern Thailand, which exceeds the standard. As a result, for consumer protection, producers and allied authorities must monitor, manage, and implement procedures to control water quality from production to consumer delivery. To make sure that, it is not affected by the site of production, the environment, or variations in meteorological conditions such as air temperature, rainfall, and season, among others.

Keywords: factors, affecting, bacterial contamination, water, southern Thailand

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Kritsanee Ruangsombat

Contents

	Page
Abstract (Thai version)	v
Abstract (English version)	vii
Acknowledgement	ix
Contents	x
List of Tables	xii
List of Figures	xiii
List of Symbols	xiv
Lists of Published Papers and Proceedings	xv
Reprint ware made with permission from the publishers	xvi
Chapter 1 Introduction	1
1.1 Water importance	1
1.2 Water quality problems in Thailand	2
1.3 Factors affecting water quality	4
1.4 Standards of water quality in Thailand	5
Chapter 2 Objectives	6
2.1 Objectives	6
2.2 Research framework	6
Chapter 3 Research methodology	9
3.1 Data source	9
3.2 Data collection	10
3.3 Data management	10
3.4 Statistical analysis	11
Chapter 4 Results and Discussion	12
4.1 Descriptive analysis	12
4.2 Multiple logistic regression models	16

Contents (cont.)

	Page
4.3 Evaluation of model performance	25
4.4 Discussion	27
Chapter 5 Concluding and remarks	30
5.1 Conclusion	30
5.2 Implementation and outcome	30
5.3 Limitation	31
5.4 Suggestions for further research	31
References	32
Appendix	40
Appendix 1	41
Paper 1 Average air temperature and total rainfall influence bacterial contamination in processed water in southern Thailand	
Appendix 2	56
Paper 2 Risk factors affecting the bacterial contamination in water of Thailand's Upper south 2020-2022	
Appendix 3	68
Proceeding 1 Relationship between environmental factors influencing the detection of total coliforms bacteria in drinking water in sealed containers, southern Thailand by multiple logistic regression	
Vitae	79

List of Tables

Tables	Page
Table 1 Water samples were classified based on environmental and other factors	14
Table 2 The association between determinant and outcome variables in sealed drinking water	17
Table 3 Factors affecting the detection of TCB in water exceeding the standard	18
Table 4 Factors affecting the detection of *E. coli* in water exceeding the standard	20
Table 5 Factors affecting the detection of *Salmonella* spp. in water exceeding the standard	22
Table 6 Factors affecting the detection of *S. aureus* in water exceeding the standard	22
Table 7 The risk factors influencing bacterial prevalence in water in upper south Thailand	23

List of Figures

Figures **Page**

Figure 1 Factors affecting the non-meet standard of TCB in drinking water 7
Figure 2 Factors associated with the detection 4 bacterial types non-standard 7
Figure 3 Risk factors influencing bacterial prevalence in upper south Thailand 8
Figure 4 Data source in south of Thailand 9
Figure 5 Annual air temperature between 2002-2022 in PHA 11 and PHA 12 12
Figure 6 Annual rainfall between 2002-2022 in PHA 11 and PHA 12 13
Figure 7 AUC of the multiple logistic regression models 25

List of Abbreviations and Symbols

Adj. OR	Adjusted Odds Ratio
Air temp	Air temperature
AUC	Area Under the ROC Curve
CI	Confidence Interval
DMSC	Department of Medical Sciences Center
E. coli	*Escherichia coli*
FCB	Fecal Coliforms Bacteria
mm	millimeters
PHA	Public Health Area
Ref	Reference
RMSC	Regional Medical Sciences Center
ROC curve	Receiver Operating Characteristic curve
S. aureus	*Staphylococcus aureus*
SDG	Sustainable Development Goal
TCB	Total Coliforms Bacteria
Temp	Temperature
UN	United Nations
WHO	World Health Organization
χ^2	Chi-square test
°C	Degree Celsius

Lists of Published Papers and Proceedings

List of Paper

1. Ruangsombat, K., Lim, A. Pradit, S., & Noppradit, P. (2023). Average air temperature and total rainfall influences bacterial contamination in processed water in Southern Thailand. *Applied Ecology and Environmental Research*, *21*(5), 3823-3836. DOI: http://dx.doi.org/10.15666/aeer/2105_38233836
2. Ruangsombat, K., Lim, A., Pradit, S., Cholumpai, V., & Noppradit, P. (2023). Risk factors affecting the bacterial contamination in water of Thailand's upper South 2020-2022. *Trends in Sciences*, (Accepted on 30 June 2023).

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1. Ruangsombat, K., & Noppradit, P. (2022). Relationship between environmental factors influencing the detection of total coliforms bacteria in drinking water in sealed containers, Southern Thailand by multiple logistic regression. *The 6th TICC International Conference "Next Normal Next Move towards Sustainable Development Goals"* 5th March 2022.

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Paper 1

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I am Kritsanee Ruangsombat, Ph.D. student on program Marine and coastal Resource Institute in Prince of Songkla University and I am first author in manuscript ref. 14320 "Average air temperature and total rainfall influence bacterial contamination in processed water in Southern Thailand". I would like to request permission to republish the manuscript in my thesis book.

Best regards

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Paper 2

Risk factors affecting the bacterial contamination in water of Thailand's upper South 2020-2022

[TiS] Manuscript Decision & Article Processing Charges

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To: Kritsanee Ruangsombat; Apiradee Lim; Siriporn Pradit; Varaporn Cholumpai; Prakrit Noppradit

Dear Professor Kritsanee Ruangsombat, Apiradee Lim, Siriporn Pradit, Varaporn Cholumpai, Prakrit Noppradit:

We have reached a decision regarding your submission to Trends in Sciences, "Risk Factors Influencing Bacterial Contamination in Water in Thailand's Upper South". The reviewers have no further comment. We are pleased to inform you that your manuscript is accepted for publication and is scheduled to be published in Trends in Sciences (TiS) in the Current Volume.

Manuscript URL: https://tis.wu.ac.th/index.php/tis/authorDashboard/submission/7158

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Proceeding 1

Relationship between environmental factors influencing the detection of total coliforms bacteria in drinking water in sealed containers, Southern Thailand by multiple logistic regression

TICC

Dear Miss Kritsanee Ruangsombat,
On behalf of The 6th TICC International Conference "Next Normal…Next Move towards Sustainable Development Goals", I am pleased to inform you that your submission ID **67**, titled
"A study of environment correlations influencing the detection of total coliforms bacteria of drinking water in sealed containers, southern region of Thailand by multiple logistic regression."
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Chapter 1

Introduction

1.1 Water importance

Water resources are necessary for human consumption, occupation, transportation, and enjoyment (Water Quality Management Bureau, 2014; Niyoyitungiye et al., 2020). Currently, the environment is changing. Water scarcity is exacerbated by the growth of the community, economy, and society. Human demand for water is currently increasing all over the world; for example, between 2010 and 2017, demand for drinking water climbed from 61% to 71% (Poulin et al., 2020). Water consumption in residential areas averaged 100 gallons per person per day (Office of the Health Promotion Foundation, 2012), a growth of 1% every year, and it is projected that by 2050, there will be an increase of 20-30% in current water use (Singhanat, 2020). Overall access to potable water according to a 2017 report by the United Nations (UN) and the World Health Organization (WHO), 5.3 billion people worldwide have access to clean water while 2.2 billion do not potable water, which the WHO estimates that 40% of the world's population has access adequate for consumption water (Sudsandee et al., 2020). Bacterial contamination in water is hazardous to human health (National Water Resources Office announcement, 2019). The United Nations (UN) stated in 2015 the objective of developing sustainable water management with a policy for all countries worldwide to give all citizens access to clean, safe, and inexpensive water to establish a sustainable system by 2030 under Sustainable Development Goals 6 or SDG6 (Alshomali and Gulseven, 2020). It is consistent with Thailand's 20-year national policy (2018-2037) to expand and develop water resource management based on the Sufficiency Economy Philosophy to create a balance between conservation, restoration, and sustainable use of water resources (National Water Resources Office announcement, 2019).

Regarding Thailand, when water quality was compared by region, the southern region was found to have acceptable to good quality, which was better than other regions (Regional Environment Office 15, 2019). In the south of Thailand, demand for water increased between 2017 and 2019. As the urban population grows and the tourism industry expands, a comprehensive evaluation of the water management plan is a long-term and cost-effective solution to the water shortage problem (Office of the National Economic and Social Development Council, 2019).

1.2 Water quality problems in Thailand

Thailand's water quality issues have deteriorated differently in each region continually, especially microorganisms, such as north of Thailand, the quality of potable water from 2010 to the present was judged to be above standard. It was unsuitable for drinking water because it was particularly polluted with Total Coliform Bacteria (TCB) (Kudsong et al., 2010). Water quality in sealed bottles containers marketed in Nan province in 2014 revealed TCB and Fecal Coliform Bacteria (FCB) at 9.3% higher than the criterion (Nimrat and Vuthiphandchai, 2014). An assessment of the quality of tap water in Phichit province in 2021 found that FCB exceeded the norm by 90% in raw water and 70% in water going through the waterworks system. As a result, the Phichit Provincial Public Health Office has recommended improvement in water quality prior to consumption for consumer safety (Phonprasertsri et al., 2021).

The central region of Thailand has problems on the water quality used for consumption in the river by residents of Bang Look Sua sub-district communities was conducted in 2015, Ongkharak District, Nakhon Nayok province, TCB discovered in water sources surpassed the acceptable criteria, which examined statistically was found to be substantially linked with diarrhea and skin disease incidence (Phakham et al., 2015). In the same year, a diarrhea pandemic was discovered, in Prachuap Khiri Khan Province, 55 patients were discovered in military schools of servicemen and kids in a school of 1,330 individuals. This was caused by drinking water infected with *E. coli* (Komol et al., 2018). In Ayutthaya province, a study was conducted in 2017, the total number of bacteria discovered, and TCB contamination above the benchmark by 40.0% and 17.5%, respectively (Kokaew et al., 2017). In 2018 the quality of potable tap water in Bangkok, Nonthaburi, and Samut Prakan was conducted by collecting data from 2010 to 2016 and comparing it to the standard, *E. coli* contamination was identified in filtered water, according to WHO regulations. Found contamination at the filtered water, dispenser, and water from tanks, at 96.71, 95.63, and 90.88%, respectively (Kordach et al., 2018).

Eastern Thailand, in 2016, a survey was conducted on the quality of treated tap water in the village of Dong Bang Sub-district. TCB contamination in Prachantakham district, Prachinburi province, exceeding the standard (Sriket et al., 2016). In 2020, an examination of the quality of ice sold near Burapha University Chonburi Province. TCB and *E. coli* contamination was found to be 36.67% (Homthong and Chumpinich, 2020). The microbiological water quality from vending machines was examined in 2016. Later in 2021, a survey on drinking water quality through filter machines in Chonburi provincial elementary schools discovered that 48.5 percent of TCB did not meet the norm, particularly at the drinking water dispensing points. The primary source of pollution is lacking in the upkeep and repair of drinking water filtration systems (Asa et al., 2021).

Southern region of Thailand, in 2012, an ice quality evaluation in the three southern border provinces of Pattani, Yala, and Narathiwat discovered that 95% failed the microbiological criterion, with 92.5%, 57.5%, and 2.5% of TCB, *E. coli*, and *Clostridium perfringens* detected, respectively (Yodmanee et al., 2012). In 2016, TCB and FCB were discovered to be noncompliant with the standard in the municipality of Yala province accounting for 25 and 10.71%, respectively (Srisuk and Phalachai, 2016). In 2018, water quality in the

Phang Nga provincial aquaculture business was reported to be 94.8%, 79.2%, and 78.1% above requirements for TCB, FCB, and *E. coli*, respectively (Jeamsripong et al., 2018). And in 2022, the contamination of culture water with *E. coli* has decreased. It was discovered that 6.7% detected *E. coli* in Phang Nga province and 3.3% in Surat Thani province, indicating that the water quality has improved (Jeamsripong et al., 2022).

1.3 Factors affecting water quality

The expansion and survival of bacteria were affected in nature by changes in the environment such as humidity, temperature, radiation, nutrition, acid-base, and air basic components (Wiwat, 1983; Jang et al., 2017). Rainfall (Songsak et al., 2017; Islam et al., 2017) and relative humidity (Suantubtim and Gamnarai, 2018; Stanaszek-Tomal, 2020) have also been reported to influence bacterial growth and survival (Hou et al., 2017; Islam et al., 2017). In 2019, Herridge et al. studied environmental factors important for microbiological water quality using FCB and *E. coli* as indicators bacteria of water quality in two rivers in Germany and found that water temperature and rainfall influence the distribution of bacteria (Herrig et al., 2019).

According to past water quality monitoring data from government laboratories in southern Thailand, which it was discovered that meteorological and spatial data such as types of water, years of production, temperature, rainfall, population, livestock utilization, and agricultural land make a difference in water quality (Poulin et al., 2020), which can have an impact on the quality of biological water over-detection of bacteria was associated with water-borne disease outbreaks and it was a major public health (Jufri, 2020). Therefore, the association between environmental conditions influencing bacterial contamination, which was investigated in southern Thailand, divided bacteria indicators and pathogenic bacteria found in clean water. It was critical to develop a predictive model for detecting microorganisms that do not meet the standard, and action should be performed immediately. Because no agency has used such information to explain the relationship's conformance or the possibility of finding microorganisms above standards. Consequently, this research provides an initial guide for every organization to recognize the importance of data that could possibly be brought integrated to improve water quality clean, and safe for consumers. This involves being able to produce a manual, clarify that and warn about hazards associated with water as the environment changes.

1.4 Standards of water quality in Thailand

Water quality regulation in Thailand has diverse requirements depending on the type of water and application, which can be categorized into three major groups: 1) Physical: color, turbidity, sediment, odor, taste, temperature, and pH. 2) Chemicals include arsenic, lead, mercury, fluoride, nitrate, iron, and pesticides. And 3) Biological including bacterial contamination. All reference to Notification of the Ministry of Public Health no. 61 (B.E. 2524): Drinking water in sealed containers, Announcement of the Department of Health B.E. 2560: Drinking tap water, Notification of the Ministry of Public Health No. 78 (B.E. 2527): Ice, and Notification of the Ministry of Public Health, No. 416 (B.E. 2563): Quality or standards, criteria, conditions, and analysis methods for pathogenic microbial foods.

Chapter 2

Objectives

2.1 Objectives

1. To investigate the impact of production location, year of production, and air temperature on TCB contamination exceeding regulations in sealed containers of drinking water in southern Thailand

2. To explore the associations between air temperature, rainfall, years of production, and four types of bacterial contamination (TCB, *E. coli*, *S. aureus*, *Salmonella* spp.) in drinking, ice, consumption, and processed water that did not meet notification of Ministry of Public Health criterion

3. To determine the risk factors that influence the prevalence of bacteria did not meet the standard in Thailand's upper southern region

2.2 Research framework

Secondary data were categorized based on the study objectives, which included: 1) TCB contamination in drinking water from non-standard sealed containers was investigated at four different sites (RMSC 11, RMSC 11/1, RMSC 12, RMSC 12/1) over six years of production (2015-2020), as illustrated in **Figure 1**. 2) The examination of the association of four bacteria contaminations (TCB, *E. coli*, *S. aureus*, *Salmonella* spp.) in two Public Health Areas (PHA 11 and PHA 12) during a 20-year period (2002-2021) with data on air temperature and rainfall in four water types consisting of drinking, ice, processed, and consumption water as shown **Figure 2**. 3) The risk factors influencing the prevalence of non-conforming bacteria in Thailand's upper southern region (7 provinces) was researched for three years, from 2020 to 2022, categorizing water types into four types, bacteria into four species, air temperature levels and precipitation levels as shown in **Figure 3**. The R programs employed statistical analysis to investigate the association between variables, data were cleaned and managed before analysis. Frequency, percentage, mean, standard deviations and graphs were employed as descriptive statistics. The chi-square test and 95% CI of odds ratios (95% CI OR) were used to determine the association between dependent variable and independent variables. And a multiple logistic regression model was employed to predict the probability of detecting bacterial contamination above the standards (R Core Team, 2020).

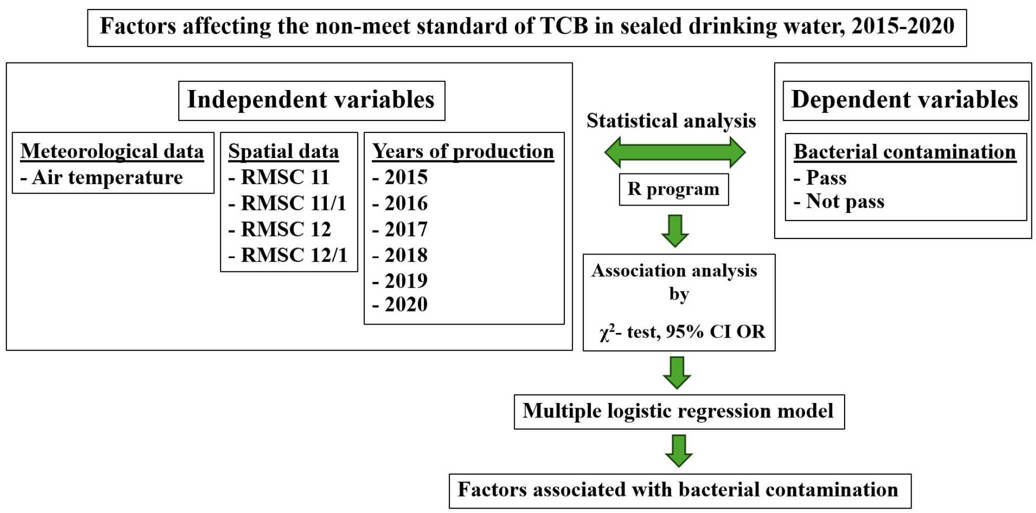

Figure 1 Factors affecting the non-meet standard of TCB in drinking water

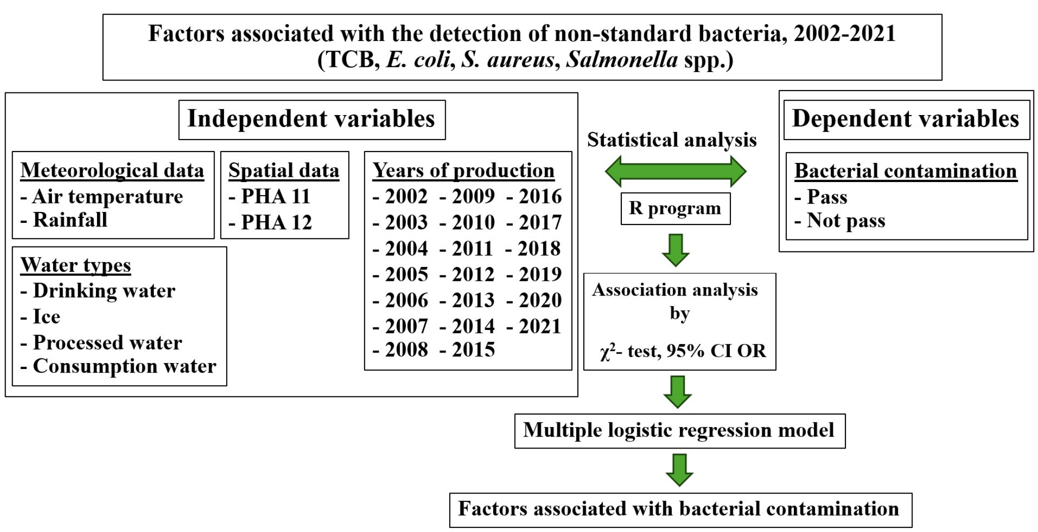

Figure 2 Factors associated with the detection 4 bacterial types non-standard

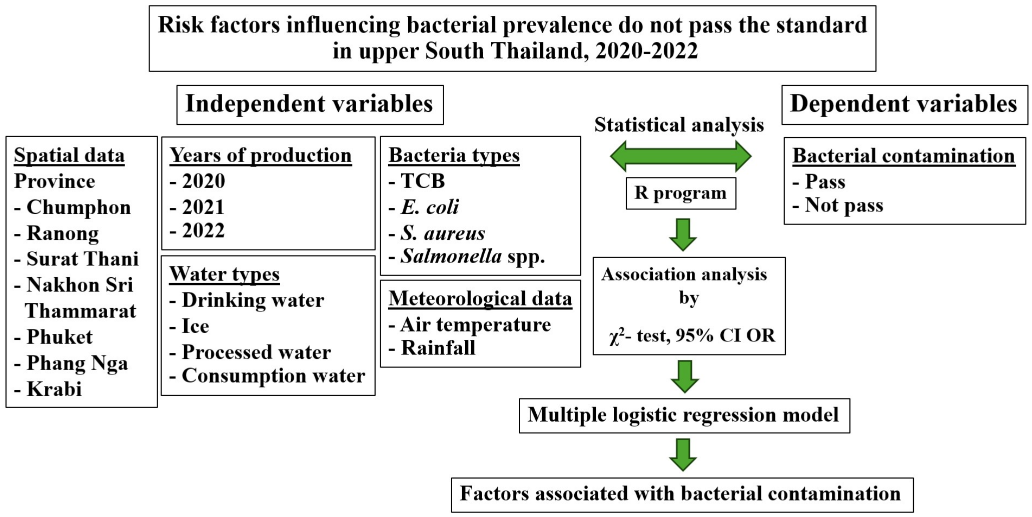

Figure 3 Risk factors influencing bacterial prevalence in upper south Thailand

Chapter 3

Research methodology

3.1 Data source

The data were derived from two sources: water quality data from annual reports from four Regional Medical Sciences Centers (RMSC): RMSC 11 (4 provinces: Chumphon, Ranong, Surat Thani, Nakhon Sri Thammarat), RMSC 11/1 (3 provinces: Phang Nga, Phuket, Krabi), RMSC 12 (4 provinces: Songkhla, Pattani, Yala, Narathiwat), and RMSC 12/1 (3 provinces: Trang, Phatthalung, Satun). The provinces were grouped into 2 public health zones: Public Health Area (PHA) 11 or upper south Thailand and PHA 12 or lower south of Thailand. The meteorological data were from the National Statistical Office of each province (14 provinces of south of Thailand), as shown in **Figure 4**.

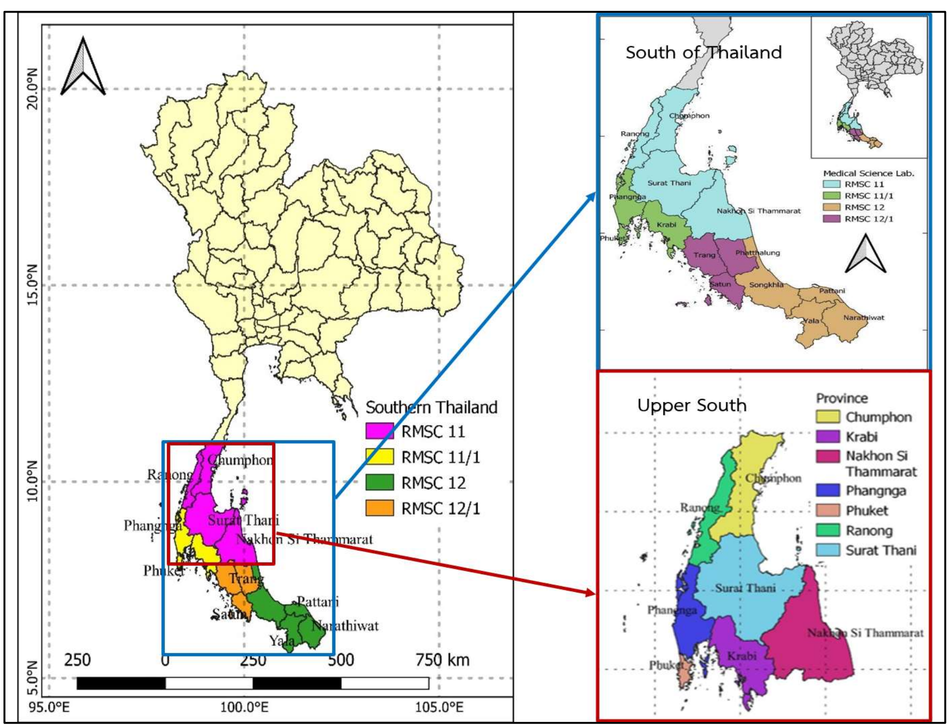

Figure 4 Data source in south of Thailand

3.2 Data collection

Secondary data from Annual Reports from two sources were as follows: 1) Bacterial water quality data: Count the total number of water samples analyzed, all samples were delivered from the product owner for quality assurance, packed in plastic bottles, plastic bags, or glass bottles, and stored in adequate conditions to minimize bacterial contamination before examination in four regional Department of Medical Science laboratories (RMSC 11, RMSC 11/1, RMSC 12, RMSC 12/1) and the number of water samples that did not satisfy standards by referring to the criteria of Notification of Ministry of Public Health No. 61 (B.E. 2524) regarding drinking water in sealed containers, Notification of the Ministry of Public Health No. 78 (B.E. 2527) Subject: Ice, and Notification of the Ministry of Public Health, No. 416 (B.E. 2563) regarding the determination of quality or standards terms and conditions and techniques for the analysis of food for harmful microorganisms. 2) The two most important meteorological variables were air temperature (°C) and total rainfall (mm) from yearly statistics of the National Statistical Office. During fiscal year 2002 to fiscal year 2022, there were a total of 21 years.

3.3 Data management

Water quality data were divided into 2 groups based on their public health areas: PHA 11 and PHA 12. Department of Medical Science laboratories 4 regionals: RMSC 11, RMSC 11/1, RMSC 12, and RMSC 12/1. The province of upper South was divided into seven provinces: Chumphon, Ranong, Surat Thani, Nakhon Sri Thammarat, Phuket, Phang Nga, and Krabi. Years of production consist of 21 years: 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017, 2018, 2019, 2020, 2021, and 2022. Type of bacteria classified into four types: TCB, *E. coli*, *S. aureus*, and *Salmonella* spp. Meteorological data were collected by the health area zones and each province similarly with water quality data. The air temperature levels were classified using the mean as a criterion. Which can be divided into two categories: less than or equal to 27.5 °C and greater than 27.5 °C. The amount of precipitation was divided into two categories based on the reference mean: less than or equal to 2,000 mm and more than 2,000 mm. PHA, province, years of production, types of bacteria, air temperature levels, and rainfall levels were all identified as independent variables in the study. Water quality contaminated with bacteria that did not meet regulations was the dependent variable.

3.4 Statistical analysis

All data were analyzed using R program version 4.1.1. Data summary and descriptive statistics were computed using EpiDisplay package. While thematic maps were created using maptools, gpclib, and the tmap packages. The descriptive analysis comprised the average and percentage. The association between independent and dependent variables was investigated by a chi-square test at a 95% confidence level. A statistical model has been developed applying multiple logistic regression models to predict the probability of bacterial detection not passing the standard. The modeling method is divided into six steps. The first step was to create a simple logistic regression model to explore the association between dependent and independent variables. Second, create an initial multiple logistic regression model using all statistically significant predictors ($p < 0.05$) from step 1. Third, select and include the appropriate independent variables in the model using the backward-direction approach. Fourth, create final multiple logistic regression models. Fifth, the final model was assessed using a Receiver Operator Characteristic curve (ROC curve), which expressed the estimated value as the Area Under the Curve (AUC), where the appropriate value should be close to 1 and greater than 0.6. Finally, interpret the model's prediction results employing Adjusted Odds Ratios (Adj OR).

Chapter 4

Results and Discussion

4.1 Descriptive analysis

Meteorological data

From 2002 to 2022, the average annual air temperature in southern Thailand ranged between 25.4 °C and 30.4 °C, with PHA 12 having a higher average temperature than PHA 11. According to the public health area, the average temperature at RMSC 11 was 27.18 ± 0.26 °C. The maximum air temperature in 2022 was 27.7 °C, and the lowest was 26.6 °C in 2011. The average temperature RMSC 11/1 was 28.67 ± 0.32 °C, the highest in 2019 was 29.3 °C and the lowest in 2022 was 27.7 °C. RMSC 12 was 28.53 ± 1.16 °C, the highest in 2004 was 30.4 °C, and in 2006 the lowest was 25.4 °C. And RMSC 12/1 was 27.53 ± 0.25 °C, the highest in 2016 was 28.1 °C, and in 2008 the lowest was 27 °C, as shown in **Figure 5**.

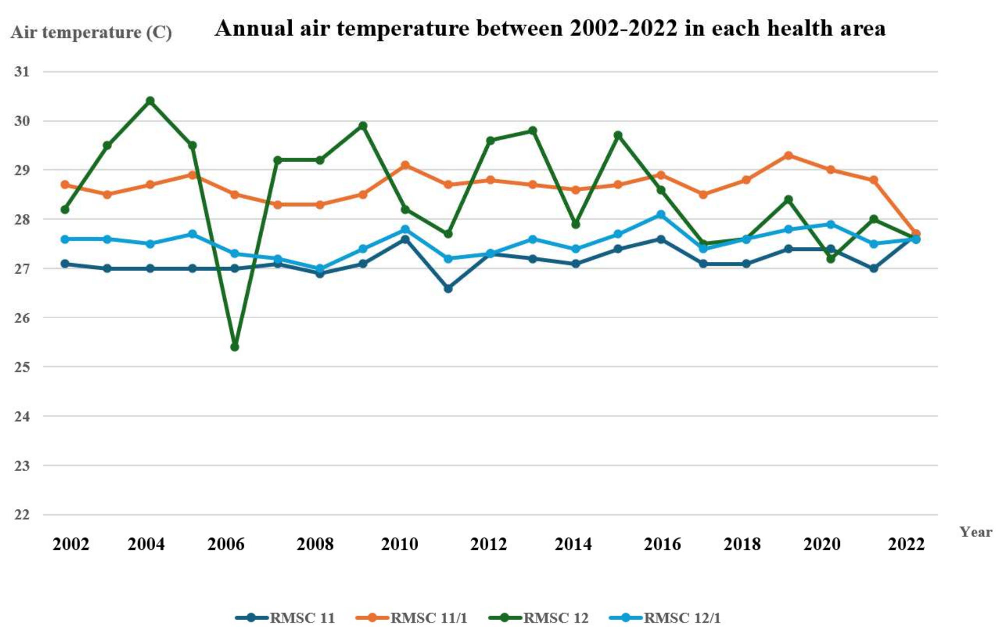

Figure 5 Annual air temperature between 2002-2022 in PHA 11 and PHA 12

The amount annual quantity of rainfall in southern Thailand was in the range of 1,112 - 3,435 mm, with PHA 12 receiving more total rainfall than PHA 11. Categorized by area, RMSC 11 had total rainfall of 1,654.6 ± 338.9 mm, the maximum total rainfall in 2022 was 2,562 mm, while the lowest was 1,112 mm in 2002. RMSC 11/1 had total rainfall of 2,315.2 ± 342.7 mm, the maximum total rainfall in 2014 was 2,827 mm, while the lowest was 1,517 mm in 2005. RMSC 12 had total rainfall of 2,302.6 ± 607.2 mm, the maximum total rainfall in 2017 was 3,435 mm, while the lowest was 1,400 mm in 2002. And RMSC 12/1 had total rainfall was 2,187.8 ± 345.4 mm, the maximum total rainfall in 2017 was 3,222 mm, while the lowest was 1,638 mm in 2002, as shown in **Figure 6**.

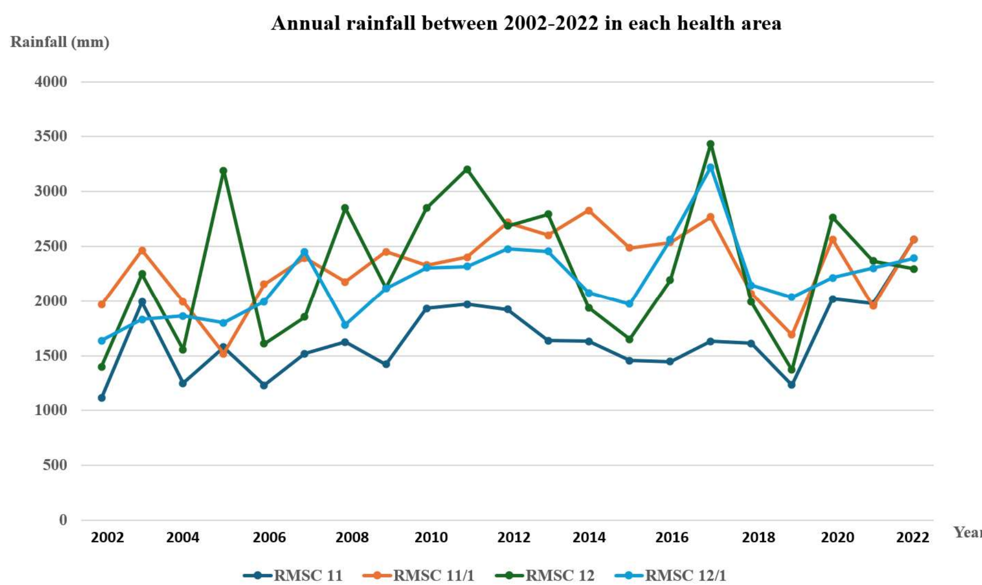

Figure 6 Annual rainfall between 2002-2022 in PHA 11 and PHA 12

Biological water quality data

The number of water samples analyzed for microbial quality from 2002 to 2022, a total of 21 years, 191,332 samples, and 7,853 samples regarded to not pass the standard (4.10%). When data was classified by PHA, RMSC, years of production, kinds of water, types of bacteria, air temperature level, and rainfall level, PHA 11 had the greatest standard water not pass (63.24%). RMSC 11 had the greatest standard water does not pass (41.64%) and RMSC 12/1 had the lowest (8.63%). The drinking water quality was found to be the most not pass the standard (45.64%), while ice met the lowest (15.01%). TCB was the most prevalent in water (74.90%), whereas *Salmonella* spp. was the least prevalent (1.32%). The bacterial contamination was highest when the air temperature was higher than 27.5 °C (60.91%) and the rainfall was less than or equal to 2,000 mm (55.39%). The water quality higher than standard in 2019 was the highest (11.45%), while that was the lowest (0.83%) in 2008, as shown in **Table 1**.

Table 1 Water samples were classified based on environmental and other factors

Factors	Samples		
	Total	Pass (%)	Not pass (%)
PHA			
PHA 11	109,024	104,058 (56.71)	4,966 (63.24)
PHA 12	82,308	79,421 (43.29)	2,887 (36.76)
RMSC			
RMSC 11	61,156	57,886 (31.55)	3,270 (41.64)
RMSC 11/1	47,868	46,172 (25.17)	1,696 (21.60)
RMSC 12	55,736	53,527 (29.17)	2,209 (28.13)
RMSC 12/1	26,572	25,894 (14.11)	678 (8.63)
Water types			
Drinking	89,388	85,804 (46.77)	3,584 (45.64)
Ice	16,468	15,289 (8.33)	1,179 (15.01)
Processed	37,820	36,560 (19.26)	1,260 (16.05)
Consumption	47,656	45,826 (24.98)	1,830 (23.30)

Table 1 Water quality is classified based on environmental and other factors (Cont.)

Factors	Samples		
	Total	Pass (%)	Not pass (%)
Bacterial types			
TCB	47,833	41,951 (22.86)	5,882 (74.90)
E. coli	47,833	46,108 (25.13)	1,725 (21.97)
S. aureus	47,833	47,691 (25.99)	142 (1.81)
Salmonella spp.	47,833	47,729 (26.01)	104 (1.32)
Air temp (°C)			
≤ 27.5	62,284	59,214 (32.27)	3,070 (39.09)
> 27.5	129,048	124,265 (67.73)	4,783 (60.91)
Rainfall (mm)			
≤ 2,000	97,300	92,950 (50.66)	4,350 (55.39)
> 2,000	94,032	90,529 (49.34)	3,503 (44.61)
Years of production			
2002	3,136	2,960 (1.61)	176 (2.24)
2003	4,128	3,873 (2.11)	255 (3.25)
2004	3,800	3,602 (11.93)	198 (2.52)
2005	5,264	5,061 (2.76)	203 (2.58)
2006	1,804	1,722 (0.94)	82 (1.04)
2007	4,412	4,241 (2.31)	171 (2.18)
2008	1,896	1,831 (0.10)	65 (0.83)
2009	6,488	6,114 (3.33)	374 (4.76)
2010	4,688	4,567 (2.49)	121 (1.54)
2011	5,056	4,881 (2.66)	175 (2.23)
2012	6,836	6,591 (3.59)	245 (3.12)
2013	12,384	12,023 (6.55)	361 (4.60)

Table 1 Water quality is classified based on environmental and other factors (Cont.)

Factors	Samples		
	Total	Pass (%)	Not pass (%)
Years of production			
2014	6,800	6,642 (3.62)	158 (2.01)
2015	15,292	14,601 (7.96)	691 (8.80)
2016	19,052	18,192 (9.92)	860 (10.95)
2017	16,340	15,568 (8.48)	772 (9.83)
2018	18,540	17,652 (9.62)	888 (11.31)
2019	17,556	16,657 (9.08)	899 (11.45)
2020	11,344	10,878 (5.93)	466 (5.93)
2021	14,652	14,315 (7.80)	337 (4.29)
2022	11,864	11,508 (6.27)	356 (4.53)

The chi-square test was used to analyze the association between the environmental and other factors (PHA, RMSC, water types, bacterial types, temperature levels, rainfall levels, and years of production,) and the detection bacterial contamination exceeds the standard at the 95% confidence level, show that all factors had a statistically significant effect on the identification of bacterial contamination that exceeded the stated criterion ($p < 0.001$).

4.2 Multiple logistic regression

Between 2015 and 2020, an examination of the impact of manufacturing site, year of manufacture, and air temperature on the detection of non-standard TCB in 10,665 samples of sealed drinking water discovered that not passed 1,795 samples, PHA and years of manufacture were factors associated with higher than the normative detection of TCB contamination in sealed packaged drinking water, PHA 11 and PHA 11/1 were 2.5 and 2.4 times more likely to not pass the criteria, respectively than PHA 12. When the years of production was considered, it was shown that TCB contamination was 1.38 and 1.34 times higher in 2015 and 2020, respectively than in 2018. While there was no statistically significant relationship between air temperature and TCB exceeding the standard, as shown in **Table 2**.

Table 2 The association between determinant and outcome variables in sealed drinking water

Factors	Samples		Adj OR	95% CI	*p*-value
	Total	Not pass			
PHA					
RMSC 11	3,932	922	2.50	(2.06,3.02)	< 0.001
RMSC 11/1	1,070	241	2.39	(1.97,2.90)	< 0.001
RMSC 12	2,856	314	1.00		
RMSC 12/1	2,807	318	1.06	(0.89,1.27)	0.495
Years of production					
2015	2,098	407	1.38	(1.12,1.69)	0.003
2016	2,244	403	1.29	(0.96,1.74)	0.096
2017	1,735	252	1.05	(0.87,1.27)	0.608
2018	2,007	277	1.00		
2019	1,335	235	1.21	(0.94,1.57)	0.144
2020	1,246	221	1.34	(1.07,1.67)	0.010
Air temp (°C)	10,665	1,795	1.03	(0.63,1.69)	0.894

The association between air temperature, rainfall, and year of production to four types of bacteria discovered to be non-compliant in four types of water researched between 2002 and 2022 deploying a total of 44,867 samples, found not pass the standard 5,564 samples, which has been identified, PHA, years of manufacture, types of water, air temperature, and rainfall levels were significant factors affecting TCB and *E. coli* contamination in water with statistical significance. The likelihood of TCB contamination was discovered to be higher in PHA 11 than in PHA 12 at 1.45 times, particularly in water generated in 2002, 2006, and 2009, which were identified as the top three most polluted waters, When the detection of TCB contamination was compared to 2021, it was discovered that between 2002 and 2009, the higher was 4.54, 2.97, 3.17, 1.84, 4.07, 1.75, 1.53, and 3.28 times, respectively, during 2011 and 2014 to 2020 higher than 1.54, 1.41, 2.02, 2.15, 2.56, 2.71, 2.27, and 1.68 times, respectively. Processed water was compared to drinking, ice, and consuming water, the TCB was found to be higher than 1.80, 2.34, and 1.52 times, respectively. At air temperatures above 28 °C, TCB contamination higher than air temperature less than or equal to 28 °C was 1.32 times. Furthermore, when the amount of precipitation surpasses 2,000 mm, TCB exceeds the standard higher 1.35 times when compared to lower rainfall levels, as shown in **Table 3**.

Table 3 Factors affecting the detection of TCB in water exceeding the standard

Factors	Samples		Adj OR	95% CI	*p*-value
	Total	Not pass			
PHA					
RMSC 11	24,710	3,251	1.45	(1.34,1.57)	< 0.001
RMSC 12	20,157	2,313	1.00		
Years of production					
2002	784	141	4.54	(3.46,5.95)	< 0.001
2003	1,032	196	2.97	(2.39,3.69)	< 0.001
2004	950	149	3.17	(2.40,4.19)	< 0.001
2005	1,316	156	1.84	(1.45,2.33)	< 0.001
2006	451	68	4.07	(2.90,5.71)	< 0.001
2007	1,103	118	1.75	(1.35,2.26)	< 0.001

Table 3 Factors affecting the detection of TCB in water exceeding the standard (cont.)

Factors	Samples		Adj OR	95% CI	*p*-value
	Total	Not pass			
Years of production					
2008	474	48	1.53	(1.08,2.18)	0.02
2009	1,622	296	3.28	(2.66,4.06)	< 0.001
2010	1,172	108	1.12	(0.87,1.44)	0.37
2011	1,264	145	1.54	(1.24,1.91)	< 0.001
2012	1,709	175	1.09	(0.86,1.38)	0.46
2013	3,096	250	1.08	(0.89,1.32)	0.42
2014	1,700	127	1.41	(1.12,1.78)	< 0.001
2015	3,823	485	2.02	(1.57,2.60)	< 0.001
2016	4,763	669	2.15	(1.73,2.67)	< 0.001
2017	4,085	609	2.56	(2.19,3.00)	< 0.001
2018	4,635	639	2.71	(2.27,3.24)	< 0.001
2019	4,389	571	2.27	(1.77,2.91)	< 0.001
2020	2,836	354	1.68	(1.39,2.03)	< 0.001
2021	3,663	260	1.00		
Types of water					
Drinking	21,109	2,861	1.80	(1.65,1.96)	< 0.001
Ice	3,651	667	2.34	(2.10,2.62)	< 0.001
Processed	9,339	853	1.00		
Consumption	10,768	1,183	1.52	(1.38,1.68)	< 0.001
Air temp (°C)					
≤ 28	22,802	2,726	1.00		
> 28	22,065	2,838	1.32	(1.17,1.48)	< 0.001
Rainfall (mm)					
≤ 2,000	18,325	2,508	1.00		
> 2,000	26,542	3,056	1.35	(1.19,1.54)	< 0.001

A total of 44,867 samples were evaluated in the investigation of factors impacting the identification of *E. coli* contamination, with 1,649 samples failing to pass the criteria. PHA 11 does not meet standards 2.70 times higher than PHA 12. As discovery was compared to 2010, it was observed that from 2002 to 2009, the higher was 11.42, 5.39, 7.55, 3.99, 12.86, 6.54, 4.99, and 6.26 times, between 2011 and 2020, greater than 2.14, 2.43, 3.93, 3.46, 4.99, 4.76, 4.29, 8.36, 9.65, and 2.79 times, respectively. When drinking water was compared to ice and consuming water, that were found to be 3.75 and 1.75 times higher, respectively. While contamination was 1.46 times more at temperatures over 28 °C than at temperatures less than or equal to 28 °C. Furthermore, when the amount of precipitation exceeds 2,000 mm, *E. coli* exceeds the standard higher 1.83 times as compared to lesser rainfall levels, as shown in **Table 4.**

Table 4 Factors affecting the detection of *E. coli* in water exceeding the standard

Factors	Samples		Adj OR	95% CI	*p*-value
	Total	Not pass			
PHA					
RMSC 11	24,710	1,172	2.70	(2.30,3.18)	< 0.001
RMSC 12	20,157	477	1.00		
Years of production					
2002	784	35	11.42	(5.55,23.48)	< 0.001
2003	1,032	50	5.39	(2.88,10.10)	< 0.001
2004	950	38	7.55	(3.76,15.14)	< 0.001
2005	1,316	32	3.99	(2.03,7.86)	< 0.001
2006	451	14	12.86	(5.44,30.43)	< 0.001
2007	1,103	45	6.54	(3.40,12.61)	< 0.001
2008	474	15	4.99	(2.32,10.74)	< 0.001
2009	1,622	58	6.26	(3.29,11.93)	< 0.001

Table 4 Factors affecting the detection of *E. coli* in water exceeding the standard (cont.)

Factors	Samples		Adj OR	95% CI	*p*-value
	Total	Not pass			
Years of production					
2010	1,172	13	1.00		
2011	1,264	28	2.14	(1.07,4.27)	0.03
2012	1,709	58	2.43	(1.32,4.48)	< 0.001
2013	3,096	94	3.93	(2.17,7.12)	< 0.001
2014	1,700	29	3.46	(1.70,7.04)	< 0.001
2015	3,823	132	4.99	(2.66,9.35)	< 0.001
2016	4,763	175	4.76	(2.62,8.65)	< 0.001
2017	4,085	150	4.29	(2.34,7.86)	< 0.001
2018	4,635	222	8.36	(4.50,15.54)	< 0.001
2019	4,389	300	9.65	(5.21,17.85)	< 0.001
2020	2,836	99	2.79	(1.56,5.02)	< 0.001
2021	3,663	62	1.39	(0.74,2.61)	0.31
Types of water					
Drinking	21,109	517	1.00		
Ice	3,651	382	3.75	(3.25,4.33)	< 0.001
Processed	9,339	330	1.08	(0.93,1.25)	0.29
Consumption	10,768	420	1.75	(1.53,2.00)	< 0.001
Air temp (°C)					
≤ 28	22,802	745	1.00		
> 28	22,065	904	1.46	(1.16,1.85)	< 0.001
Rainfall (mm)					
≤ 2,000	18,325	875	1.00		
> 2,000	26,542	774	1.83	(1.44,2.33)	< 0.001

A total of 44,867 samples were collected and 99 samples failed the standard. Factors related to the detection of *Salmonella* spp., as well as which PHA and water types reveal statistically significant contamination in the water, with PHA 11 being 4.34 times more likely to contaminate than PHA 12. When compared to drinking water, the contamination was greater in ice, processed, and consuming water, 2.76, 2.87, and 4.30 times respectively, as shown in **Table 5**.

Table 5 Factors affecting the detection of *Salmonella* spp. in water exceeding the standard

Factors	Samples		Adj OR	95% CI	*p*-value
	Total	Not pass			
PHA					
RMSC 11	24,710	81	4.34	(2.45,7.68)	< 0.001
RMSC 12	20,157	18	1.00		
Types of water					
Drinking	21,109	22	1.00		
Ice	3,651	12	2.76	(1.35,5.67)	< 0.001
Processed	9,339	30	2.87	(1.60,5.14)	< 0.001
Consumption	10,768	35	4.30	(2.44,7.58)	< 0.001

Similarly, a total of 44,867 samples were used to determine factors causing the detection of *S. aureus*, with 135 of these not passing the criteria. The detection of *S. aureus* contamination had only water categories that were statistically substantially, when compared to drinking water found ice, processed water, and ingested water were demonstrated to have a 17.82, 2.85, and 5.28 times higher not pass the criterion, as shown in **Table 6**.

Table 6 Factors affecting the detection of *S. aureus* in water exceeding the standard

Factors	Samples		Adj OR	95% CI	*p*-value
	Total	Not pass			
Types of water					
Drinking	21,109	19	1.00		
Ice	3,651	54	17.82	(10.48,30.29)	< 0.001
Processed	9,339	41	2.85	(1.52,5.36)	< 0.001
Consumption	10,768	21	5.28	(3.01,9.26)	< 0.001

The investigation of risk factors influencing the identification of the prevalence of non-standard bacteria in water in Thailand's upper southern region or PHA 11 between 2020 and 2022. The study comprised 6,142 water samples, 674 of which did not meet the criteria. According to the findings of the investigation, province, years of production, water types, bacterium types, and rainfall levels were risk factors influencing bacterial contamination exceeding the standard in PHA 11. When compared to Phuket, the provinces of Chumphon, Surat Thani, Ranong, Nakhon Sri Thammarat, and Krabi exhibited higher rates of detectable bacteria contamination by 3.35, 1.93, 2.28, 2.56, and 2.91 times, respectively. Water generated in 2022 was 1.45 times more likely to fail to fulfill the criteria than in 2021. When compared to drinking water, consumption, ice, and processed water quality were at risk of exceeding the standard 3.41, 2.40, and 2.79 times, respectively. TCB, *E. coli*, and *S. aureus* were revealed that 40.47, 13.46, and 2.79 times more than *Salmonella* spp., respectively. And bacterial contamination increases as total rainfall more than 2,500 mm was 1.78 times, as shown in **Table 7**.

Table 7 The risk factors influencing bacterial prevalence in water in upper South Thailand

Factors	Samples		Adj OR	95% CI	*p*-value
	Total	Not pass			
Province					
Phuket	2,450	212	1.00		
Chumphon	365	53	3.35	(2.27,4.93)	< 0.001
Surat Thani	1,484	149	1.93	(1.46,2.56)	< 0.001
Ranong	158	33	2.28	(1.36,3.80)	0.001
Nakhon Sri	742	122	2.56	(1.85,3.54)	< 0.001
Phang Nga	490	56	1.46	(0.98,2.16)	0.062
Krabi	453	49	2.91	(1.91,4.43)	< 0.001
Years of production					
2020	1,530	157	1.19	(0.83,1.71)	0.338
2021	2,066	205	1.00		
2022	2,546	312	1.45	(1.09,1.94)	0.010
Types of water					
Drinking	2,635	167	1.00		
Ice	915	109	2.40	(1.81,3.20)	< 0.001
Processed	169	24	2.79	(1.68,4.63)	< 0.001
Consumption	2,423	374	3.41	(2.75,4.23)	< 0.001
Bacterial types					
TCB	1,949	473	40.47	(22.09,74.13)	< 0.001
E. coli	1,625	161	13.46	(7.26,24.98)	< 0.001
S. aureus	1,274	29	2.79	(1.38,5.61)	0.004
Salmonella spp.	1,294	11	1.00		

Table 7 The risk factors influencing bacterial prevalence in water in upper South Thailand (cont.)

Factors	Samples		Adj OR	95% CI	*p*-value
	Total	Not pass			
Rainfall (mm)					
≤ 2,500	3,808	379	1.00		
> 2,500	2,334	295	1.78	(1.27,2.5)	< 0.001

Note: Nakhon Sri = Nakhon Sri Thammarat

4.3 Evaluation of model performance

The predictive performance of the multiple logistic regression models by comparing the values obtained from the model and the true values from the observation to create a graph with the sensitivity on the y-axis and the false positive (1-specificity) on the x-axis and measuring the area under the curve (AUC). Overall, the AUC was found to be in the range of 0.61 – 0.82 as shown in **Figure 7**, that the models developed were in good concord with the real data. Predicted values were accurate and correct. The AUC of the model predicting higher-than-standard TCB detection in sealed bottled water was 0.63 (**Figure 7** (a)). Prediction models for bacterial contamination over the threshold classified by bacterial strains, TCB, *E. coli*, *Salmonella* spp. and *S. aureus* were 0.61, 0.68, 0.78, and 0.81, respectively (**Figure 7** (b), (c), (d), (e)). The AUC for the bacterial prevalence prediction model split by the province in PHA 11 was 0.82 (**Figure 7** (f)).

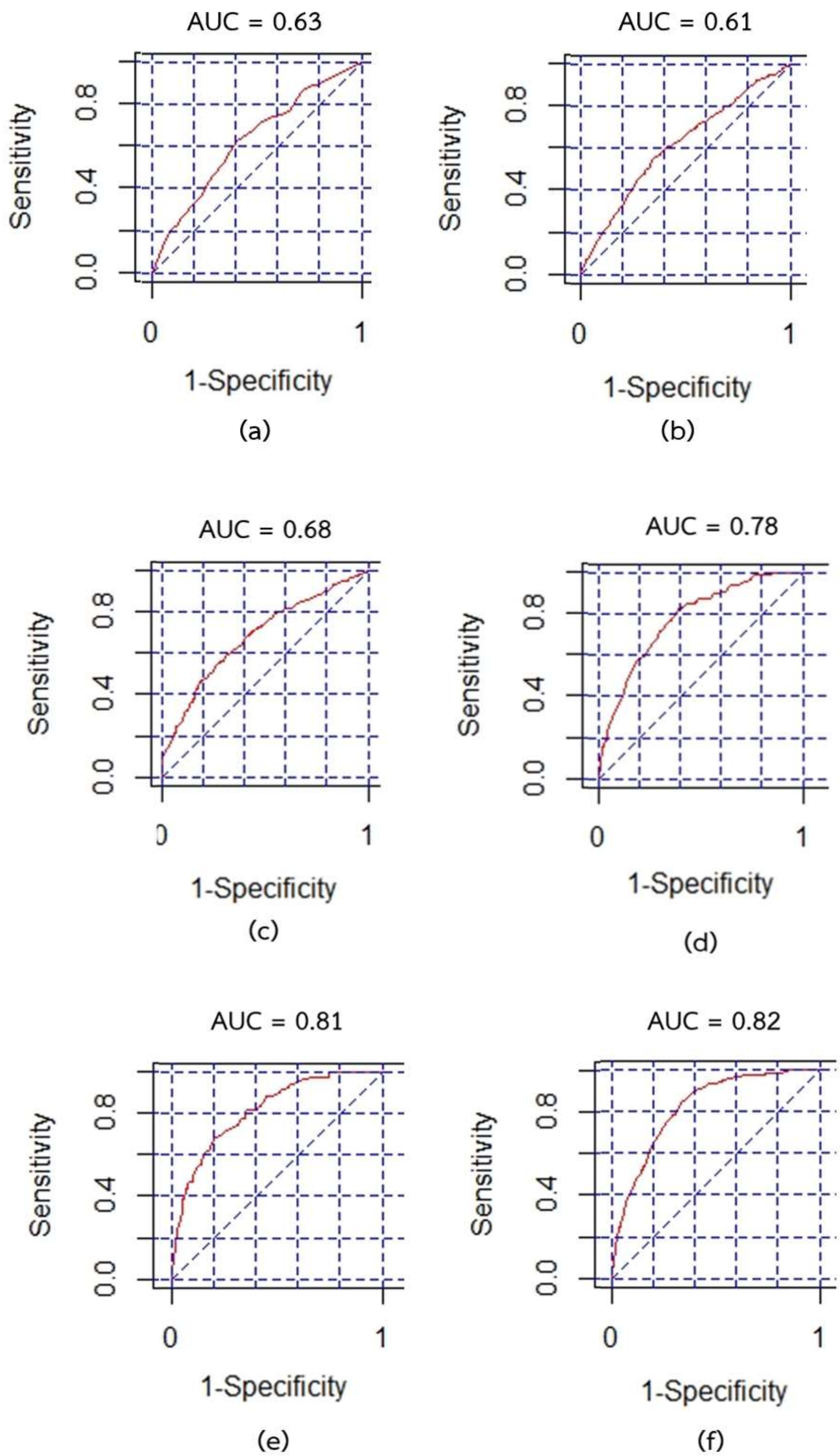

Figure 7 AUC of the multiple logistic regression models

4.4 Discussion

The investigation of the association between environmental factors and other factors contributing to bacterial contamination in water resources in southern Thailand. Using the multiplicative logistics regression model, which was discovered that the risk factors that caused the bacterial contamination to exceed the standard were the production locate, years of production, water types, bacterial species, temperature, and rainfall.

The locations and years of manufacture have an impact on bacterial contamination in water. According to the findings of this study, health zone 11 had a higher bacterial prevalence than health zone 12, where the frequency of bacteria varied from year to year. These were due to the geographical differences in production sources, which result in variances in water quality. Likewise, the study by Bastaraud et al. indicates location of site the main factor of bacterial contamination (Bastaraud et al., 2018). Abdulmana et al. revealed that changes in land use and construction elevation were factors influencing changes in geographic conditions (Abdulmana et al., 2022; Love and Laub., 2022). Production site changes have been demonstrated to have quite a large influence on climate and water quality (Powers et al., 2023). This is congruent with the findings of Archer et al., who found that climate change and geography will enhance the dispersion pathogenic bacteria (Archer et al., 2023), which affect changes in nutrients, oxygen, water activity, and pH are factors that influence bacterial behavior and survival (Gomez et al., 2020).

Bacterial contamination is affected by the kinds of water. According to the findings of this study, the highest bacterial contamination was found in consumption, followed by process, ice, and drinking water, respectively, while when classified by province, the highest bacterial contamination was found in consumption, followed by process, ice, and drinking water, respectively. This is congruent with Long et al.' s study, which found that the most important elements determining bacterial contamination are water source type (Long et al., 2016; Bastaraud et al., 2018). Pandey et al. claimed that drinking water quality must be free of bacterial contamination (Pandey et al., 2014). The criteria may differ depending on the water source: drinking water and ice are held to very high standards, however, water quality in another source may be held to less stringent standards because it is not drunk but used for recreation (LibreTexts Biology., 2023). Likewise, there must not be any bacterial contamination in the ice, which contamination can occur throughout processing, transportation, and storage. For this reason, quality control methods must be always applied to ensure the safety of ice before it reaches the consumer (Wang et al., 2023). Additionally, the research of Chalorsuntisakul and Kasornpikul showed that different types of water sources had distinct effects on bacterial contamination. They discovered that the highest bacterial contamination was found in surface water, followed by groundwater, and tap water in small beef cattle farms near Silpakorn University Phetchaburi Campus. According to the conclusion of this study, water that has undergone a rigorous disinfection procedure can assist lower the number of bacteria (Chalorsuntisakul and Kasornpikul, 2020).

The kinds of bacteria influence contamination in water. The study discovered that the indicator bacteria were more detectable than the pathogenic bacterium. TCB was the most common, followed by *E. coli*, *S. aureus*, and *Salmonella* spp. This is congruent with a study by Metz et al., which found that indicator bacteria (TCB, *E. coli*) were easier to detect than harmful bacteria (*S. aureus*, *Salmonella* spp.) (Metz et al., 2020). Which is frequently used as a predictor of the presence of pathogens in water. The discovery of these bacteria is utilized as a safety criterion for the microbiological quality of potable water (Saxena et al., 2015; Motlagh and Yang, 2019; Bivins et al., 2020; Some et al., 2021). Moreover, the cost of analysis is not expensive. The procedure is not difficult and takes less time than detecting harmful bacteria (Korajkic et al., 2018).

The air temperature affects TCB, and *E. coli* contamination and this study found that contamination levels rise as the temperature rises. While temperature, there was no effect on *S. aureus* and *Salmonella* spp. contamination in water. This may be because the indicator bacteria are more readily detected in water than pathogenic bacteria. Power et al. (2023) stated that *E. coli* contamination levels are higher at increasing temperatures due to the bacterial growth period making it more detectable, according to Hochard et al. (2023), TCB and *E. coli* concentrations increased with increasing air temperature and summer-influenced bacterial abundance. Furthermore, investigations by Xu et al. (2019), show that indicator bacteria concentrations have a substantial positive connection with air temperature.

Rainfall is a significant element in increasing microbial density. According to this study, total rainfall of more than 2,000 mm increases bacterial contamination of water sources. According to a 2017 study by Topalcengiz et al. (2017), rain runoff washes sewage from residences into water sources contaminates the water, and increases microorganism densities, particularly indicator bacteria. Similarly, Rowles et al. (2020), discovered that TCB and *E. coli* concentrations increased considerably with increasing rainfall or after heavy rainfall in a 2020 study conducted in Bangladesh and Cambodia. The Love and Laub study in Texas showed TCB concentrations were much greater than *E. coli* concentrations when rainfall rose, which was notably true in cities rather than rural regions (Love and Laub, 2022). In addition, Powers et al. (2023), conducted a study of drinking water in Kenya and found that the quality of drinking water held in houses during heavy rains for 7 days resulted an increase *E. coli* contamination identified.

Chapter 5

Concluding and remarks

5.1 Conclusion

According to this study, the multivariate logistic regression model was consistent with the data in the range of 0.61-0.82, indicating that the generated model had relatively good performance prediction accuracy. It was discovered that environmental and spatial factors had an association with bacterial contamination in water in southern Thailand that exceeded the standard, which could happen at any time. Especially during heavy rains and the rainy season, in all types of water sources, and at every production site. Indicator bacteria are easier to identify than pathogen microorganisms. Their detection has been associated with climate change and nutrient abundance. Water quality for drinking and consumption must be clean, safe, and devoid of hazardous microbes. The quality of the ice packed in plastic bags, which are readily destroyed, is of special concern. As a result, should take extra measures. Because the chances of finding a pathogenic bacterium (*S. aureus*) are quite high. Every sector should keep vigilant tabs on environmental changes, from the manufacturing process, the manufacturing site, the transportation process, and the storage time till it is sold to consumers.

5.2 Implementation and outcome

The main advantage of this study is that it identifies elements that influence the detection of bacterial contamination in clean water and predicts the likelihood of detecting water quality that does not match the required criteria. Water quality changes can be managed by stakeholders. Control dangers, prevent and monitor the spread of water-borne diseases, and reduce public health issues. This data can be used to consider corporate management's policy decisions to make plans for future operations. In addition, all organizations can be integrated and collaborate effectively to solve long-term water quality problems.

The association between environmental conditions and bacterial pollution in water has never been studied before in Thailand's southern coastal region. As a result, the findings of this study serve as a beginning for raising awareness among key agencies about the importance of data and working collaboration to maintain good water quality. Consumers are confident in the quality of their water to consume. This includes being able to establish recommendations for the prevention, surveillance, warning, communication, and risk assessment of water hazards because of climate change.

5.3 Limitation

The data used in this investigation was from secondary data with limited access. Because it will have an impact on the negative effects of stakeholders, causing some details of the information to be incomplete such as geographical data, physical and chemical water quality, antimicrobial susceptibility, times, and seasons. It was also discovered that each data organization had an individual template. That is unable to detect data mistakes and requires a long time to organize the data before bringing it to analysis.

5.4 Suggestion for further research

For further research should search for more detailed spatial data (sub-district, district, province). More information about the physical and chemical properties of water, antimicrobial susceptibility tests, as well as the seasons. Then connect to the population, morbidity, mortality, water-borne disease outbreaks in the study area, and forecast trends that occur the next time.

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Appendix

Appendix 1

Paper 1: Average air temperature and total rainfall influence bacterial contamination in processed water in Southern Thailand

AVERAGE AIR TEMPERATURE AND TOTAL RAINFALL INFLUENCE BACTERIAL CONTAMINATION IN PROCESSED WATER IN SOUTHERN THAILAND

RUANGSOMBAT, K.[1,3] – LIM, A.[2] – PRADIT, S.[1,3] – NOPPRADIT, P.[1,3*]

[1]*Marine and Coastal Resources Institute, Faculty Environmental Management, Princes of Songkla University, Hat Yai, Songkhla, Thailand*

[2]*Department of Mathematics and Computer Science, Faculty Science and Technology, Princes of Songkla University, Rusamilae, Pattani, Thailand*

[3]*Coastal Oceanography and Climate Change Research Center, Faculty Environmental Management, Princes of Songkla University, Hat Yai, Songkhla, Thailand*

**Corresponding author*
e-mail: prakrit.n@psu.ac.th

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Abstract. Testing for bacteria in water is done based on intended purposes, such as drinking, producing ice, utilizing it in the house, producing water taps, and processing water. Bacterial growth and survival in water are influenced by environmental factors, which may have consequences for human health. The purpose of this study was to identify factors influencing the failing standard of water quality for consumption. Water quality data from the annual report of Regional Medical Sciences Center and meteorological data from the National Statistical Office of Thailand were obtained for the fiscal years 2002-2021. A logistic regression model was used to identify factors associated with the failing standard of water quality for consumption. The findings revealed that 16.6% of the total sample did not meet the consumption standard, with Public Health Area (PHA) 11 and 12, failing at rates of 49.6% and 38.3%, respectively. Overall, water produced in PHA 11 was statistically ($p\text{-value} < 0.05$) substantially associated with bacterial contamination, which increased with production year, air temperature, and precipitation. In conclusion, environmental factors and other water quality were influential on biological water quality in Southern Thailand. Therefore, necessary measures must be taken to improve water quality standards in this area to safeguard the protection of consumers.
Keywords: *air temperature, rainfall, bacterial contamination, processed water, Southern Thailand*

Introduction

Water is essential for human survival in terms of consumption, employment, communication, and leisure (Bureau of Water Quality Management, 2014; Nimrat et al., 2021). Water shortage issues and water pollution, which have impacts on public health, are brought on by the current environmental changes and the growth of the community, economy, and society (Notification of National Water Resources office, 2019). Global water consumption demand for humanity has increased since 1900, and it is predicted that by 2050, water consumption will have increased by 20-30% (Singhanart, 2020). According to the United Nations (UN), 17 Sustainable Development Goals (SDGs) in 2015 were issued, and the sixth SDG is “Clean Water and Sanitation,” aiming to make clean, safe, and affordable water available to everyone on earth by 2030 (Alshomali and Gulseven, 2020). In parallel, the Thai government issued its national strategy in 2019 which covered the management of water resources (Notification of National Water Resources office, 2019). Between 2017 and 2019, water use in Southern Thailand’s

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coastline region increased. This became a factor in the raw water shortage problems over the summer because humans require more water in their daily lives while the amount of water in nature remains constant or decreases. This issue was especially brought up in urban regions and famous tourist destinations (Office of the National Economics and Social Development Council, 2022). However, Southern Thailand has better conditions than other areas in terms of water quality (Regional Environment Office 15, 2019).

Evaluation of the biological, chemical, and physical components of water can be referred to as water quality assessment (Bojarczuk et al., 2018; Jufri, 2020). Chemicals, viruses, protozoa, and parasites are all sources of contamination; however, in terms of prevalence, microbial pathogens pose the greatest threat to water safety (Badeenezhad et al., 2020). The examination of bacterial indicators in water, also known as Total Coliform Bacteria (TCB) and Fecal Coliform Bacteria (FCB), requires no special equipment and has low assay costs when compared to other bacteria (Hales, 2019; Seo et al., 2019). They can be found in the digestive systems of warm-blooded creatures, including humans. If they are found in water supplies, this implies that feces have contaminated the water (Nuangjui and Chanphiwat, 2012; Seo et al., 2019). A pathogenic bacterial indication is also present, which might result in serious conditions like gastroenteritis and diarrhea. The pathogens that cause diseases in humans, such as cholera, dysentery, and typhoid fever (*Vibrio cholerae*, *Shigella dysenteries*, *and Salmonella typhimurium* infection), can be identified using these pollutants (Kudsong et al., 2010; Nimrat et al., 2021; Takal and Quaye-Ballard, 2018). In fact, the quality standard in drinking water, ice, and processing water is set at TCB which must be less than 2.2; *Escherichia coli (E. coli)* should not be found (Notification of Public Health, 1981, 1984); *S. aureus* is less than 100 colony forming unit (CFU); and *Salmonella* spp. should not be found. Importantly, all product samples must be examined at least once a year (Notification of Public Health, 2020). Previous laboratory analysis of bacteria revealed that the water quality after passing a standard process and during delivery to consumers consistently failed to meet the specified standards. These could lead to epidemics and other public health problems. The main factors influencing bacterial growth are the environments in which they have previously existed. Water quality issues may be brought on by environmental factors such as temperature, rainfall, and poor water quality (Jufri, 2020). Moreover, water that has been improperly and unnaturally stored can have an unacceptable level of bacterial contamination (Gizaw et al., 2022), and because bacteria can constantly grow and change in number, this could lead to future health issues for consumers (Bureau of Environmental Health, 2009). To illustrate, changes in environmental factors are primarily the results of human actions, which influence climatic conditions, particularly increases in air temperature, precipitation, and degradation of water quality (Rajesh and Rehana, 2022). Therefore, some environmental experts predict that these factors have significant impacts on the environment and cause water management issues in the future (Marks, 2011; Minnesota Pollution Control Agency, 2008). Seasons, weather, temperature, distance from pollution sources, livestock management techniques, wildlife activity, excrement, sewage, and rainfall were the main environmental elements that affected bacterial contamination, growth, and survival (Islam et al., 2017; Joklik et al., 1980; Jufri, 2020; Jung et al., 2014; Minnesota Pollution Control Agency, 2008; You et al., 2019). However, when bacteria numbers fluctuate, it will be harder to detect bacterial loads, which is crucial for preventing waterborne outbreaks (Rodigues et al., 2019).

Previous water quality monitoring data from government laboratories in Southern Thailand showed that biological standards were steadily deteriorating, which had the potential to cause epidemics and they were considered a public health issue. However, there was no study of factors that influenced the detection of bacteria that was greater than the standard value and was the source of water-borne diseases in PHA 11 and 12. As a result, it must be completed as soon as possible. Therefore, the purpose of this study was to investigate the associations between environmental factors (i.e., air temperature, rainfall) and bacterial contamination in drinking water, ice, water used in households, and processed water, which did not meet the requirements in PHA 11 and 12. This study's findings could explain changes in water quality. This study's findings could explain changes in water quality, sources of bacterial contamination and assist in reducing public health problems for long-term solutions. This also includes decision-making, creating operational plans for executives at relevant department levels to solve water quality problems in an effective and sustainable manner.

Materials and methods

Data sources

Data on bacteria-contaminated water quality samples from PHA 11 (Chumphon, Ranong, Surat Thani, Nakhon Sri Thammarat, Phang-nga, Krabi, and Phuket provinces) and PHA 12 (Trang, Phatthalung, Satun, Songkhla, Pattani, Yala, and Narathiwat provinces) between fiscal years 2002 and 2021 were obtained from two Regional Medical Science Centers (RMSC) in region 11 (RMSC 11 and RMSC 11/1) and two RMSC in region 12 (RMSC 12 and RMSC 12/1) (*Fig. 1*). Water quality data included the year of manufacture, public health area, types of water, and the number of samples with bacteria contamination. Meteorological data included the average air temperature and rainfall were obtained from the National Statistical Office's annual statistics.

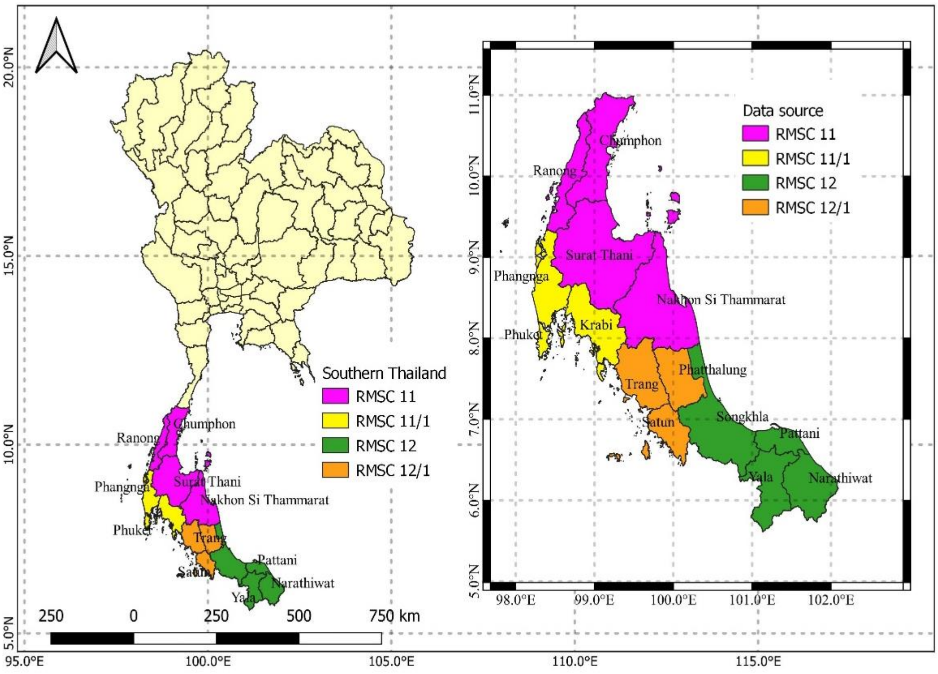

Figure 1. *Data sources in Southern Thailand; PHA 11 and PHA 12*

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The provinces include 2 PHAs in Southern Thailand: PHA 11 and PHA 12. The years ranged from 2002 to 2021. Water was classified into two groups: product water (water that can be produced in accordance with the given requirements or quality standards, for example, drinking water and ice) and processed water (water that has been chlorine-treated or basic filtered for use in washing raw materials, cooking food, and consuming), and four types: drinking, ice, consumption, and treated water (purified water undergoes a contaminant filtration process and chemical conditioning to be suitable for use, such as tap water). Water quality was divided into two groups: pass and fail from the standard of water quality for consumption. The quality was considered by looking at the contamination with bacteria such as TCB, *E. coli*, *Staphylococcus aureus* (*S. aureus*), and *Salmonella* spp. in accordance with the quality requirements set out in Ministry of Public Health Notification No. 61 (B.E. 2524), No. 78 (B.E. 2527) and No. 416. (B.E. 2563). The air temperature was divided into two groups: 26 to 28°C, and more than 28 to 29°C. Rainfall was also divided into two groups: 1000 to 2000 mm, and more than 2000 to 4000 mm.

Statistical data analyzed with the R software

All statistical analysis was done using the R program (R Core Team, 2020). Descriptive statistics were also performed. The factors associated with the failing standard of water quality for consumption were analyzed. The determinants were PHA, types of water, years of production, air temperature levels and rainfall levels. The outcomes of this study were water samples which passed or failed the standard of water quality for consumption. Chi-square test was then used to find the association between determinants and the outcomes. After that, multivariate analysis was performed using logistic regression. P-value < 0.05 was considered statistically significant, which were explained by Adjust Odds Ratio (Adj. OR) separate each factor and bacterial type. The ROC curve was used to evaluate the goodness of fit.

Results

Data regarding water quality

For the overall descriptive analysis, 16.6% of water in Southern Thailand did not meet the standard. The contamination varied according to PHA, water types, production years, air temperature, and rainfall levels. PHA 11 was the most common contaminant, accounted for 49.6% of the total, by TCB, *E. coli*, *S. aureus,* and *Salmonella* spp., which were each accounted for 63.6, 31.7, 2.5, and 2.2%, respectively. Drinking water contamination was the highest at 45.9%, with TCB, *E. coli*, *S. aureus,* and *Salmonella* spp. accounted for 51.4, 31.4, 24.1, and 22.2%, respectively. The highest level of contamination in the year of production in 2019 was 12.1%, with TCB, *E. coli*, *S. aureus*, and *Salmonella* spp. accounted for 10.3, 18.2, 14.1, and 9.1%, respectively. The air temperature of more than 28-29°C were the most common contaminant, accounted for 51.7%, by TCB, *E. coli*, *S. aureus*, and *Salmonella* spp., which accounted for 51.0, 54.8, 54.8, and 37.4%, respectively. More than 2,000 to 4,000 mm of the rainfall level fell short of the required standard by 52.93%. TCB, *E. coli, S. aureus* and *Salmonella* spp. contamination accounted for 54.9%, 46.9%, 48.2%, and 47.5%, respectively (*Table 1*).

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***Table 1**. Bacterial water quality classified by environmental factors*

Factors	Number (%)									
	Total		TCB		*E. coli*		*S. aureus*		*Salmonella* spp.	
	Pass	Failed	Pass	Failed	Pass	Failed	Pass	Failed	Pass	Failed
Public health areas										
PHA 11	21014 (46.84)	3696 (49.63)	21459 (47.83)	2351 (63.61)	23538 (52.46)	1172 (31.71)	24618 (54.87)	92 (2.49)	24629 (54.89)	81 (2.19)
PHA 12	17306 (38.57)	2851 (38.28)	17844 (39.77)	2313 (81.13)	19680 (43.86)	477 (16.73)	20114 (44.83)	43 (1.51)	20139 (44.89)	18 (0.63)
Types of water										
Drinking	17690 (47.27)	3419 (45.91)	18248 (46.42)	2861 (51.42)	20592 (47.65)	517 (31.35)	21090 (47.15)	19 (14.07)	21087 (47.10)	22 (22.22)
Making Ice	2536 (6.78)	1115 (14.97)	2984 (7.59)	667 (11.99)	3269 (7.56)	382 (23.16)	3597 (8.04)	54 (40.00)	3639 (8.13)	12 (12.12)
Consumption	8105 (21.66)	1234 (16.57)	8486 (21.59)	853 (15.33)	9009 (20.84)	330 (20.01)	9318 (20.83)	21 (15.55)	9309 (20.79)	30 (30.30)
Process water	9089 (24.29)	1679 (22.54)	9585 (24.39)	1183 (21.26)	10348 (23.94)	420 (25.47)	10727 (23.98)	41 (30.37)	10733 (23.97)	35 (35.35)
Years of production										
2002	608 (1.62)	176 (2.36)	643 (1.64)	141 (2.53)	749 (1.73)	35 (2.12)	784 (1.75)	0 (0.00)	784 (1.75)	0 (0.00)
2003	777 (2.08)	255 (3.42)	836 (2.13)	196 (3.52)	982 (2.27)	50 (3.03)	1028 (2.30)	4 (2.96)	1027 (2.29)	5 (5.05)
2004	752 (2.01)	198 (2.66)	801 (2.04)	149 (2.68)	912 (2.11)	38 (2.30)	942 (2.11)	8 (5.92)	947 (2.11)	3 (3.03)
2005	1113 (2.97)	203 (2.72)	1160 (2.95)	156 (2.80)	1284 (2.97)	32 (1.94)	1306 (2.92)	10 (7.41)	1311 (2.93)	5 (5.05)
2006	369 (0.99)	82 (1.10)	383 (0.97)	68 (1.22)	437 (1.01)	14 (0.85)	451 (1.01)	0 (0.00)	451 (1.01)	0 (0.00)
2007	932 (2.49)	171 (2.30)	985 (2.51)	118 (2.12)	1058 (2.45)	45 (2.73)	1098 (2.45)	5 (3.70)	1100 (2.46)	3 (3.03)
2008	409 (1.09)	65 (0.87)	426 (1.08)	48 (0.86)	459 (1.06)	15 (0.91)	474 (1.06)	0 (0.00)	472 (1.05)	2 (2.02)
2009	1248 (3.33)	374 (5.02)	1326 (3.37)	296 (5.32)	1564 (3.62)	58 (3.52)	1610 (3.60)	12 (8.89)	1614 (3.61)	8 (8.08)
2010	1051 (2.81)	121 (1.62)	1064 (2.71)	108 (1.94)	1159 (2.68)	13 (0.79)	1172 (2.62)	0 (0.00)	1172 (2.62)	0 (0.00)
2011	1089 (2.91)	175 (2.35)	1119 (2.85)	145 (2.61)	1236 (2.86)	28 (1.70)	1262 (2.82)	2 (1.48)	1264 (2.82)	0 (0.00)
2012	1464 (3.91)	245 (3.29)	1534 (3.90)	175 (3.14)	1651 (3.82)	58 (3.52)	1701 (3.80)	8 (5.93)	1705 (3.81)	4 (4.04)
2013	2735 (7.31)	361 (4.85)	2846 (7.24)	250 (4.49)	3002 (6.95)	94 (5.70)	3090 (6.91)	6 (4.44)	3085 (6.89)	11 (11.11)
2014	1542 (4.12)	158 (2.12)	1573 (4.00)	127 (2.28)	1671 (3.87)	29 (1.76)	1699 (3.80)	1 (0.74)	1699 (3.79)	1 (1.01)
2015	3188 (8.52)	365 (8.53)	3338 (8.49)	485 (8.72)	3691 (8.54)	132 (8.00)	3811 (8.52)	12 (8.89)	3817 (8.53)	6 (6.06)
2016	3903 (10.43)	860 (11.55)	4094 (10.42)	669 (12.02)	4588 (10.61)	175 (10.61)	4755 (10.63)	8 (5.93)	4755 (10.62)	8 (8.08)
2017	3313 (8.85)	772 (10.37)	3476 (8.84)	609 (10.94)	3935 (9.10)	150 (9.10)	4083 (9.13)	2 (1.48)	4074 (9.10)	11 (11.11)
2018	3741 (10.00)	894 (12.00)	3996 (10.17)	639 (11.48)	4413 (10.21)	222 (13.46)	4618 (10.32)	17 (12.59)	4619 (10.32)	16 (16.16)
2019	3490 (9.33)	899 (12.07)	3818 (9.71)	571 (10.26)	4089 (9.46)	300 (18.19)	4370 (9.77)	19 (14.07)	4380 (9.78)	9 (9.09)
2020	2370 (6.33)	466 (6.26)	2482 (6.32)	354 (6.36)	2737 (6.33)	99 (6.00)	2824 (6.31)	12 (8.89)	2835 (6.33)	1 (1.01)
2021	3326 (8.89)	337 (4.52)	3403 (8.66)	260 (4.67)	3601 (8.33)	62 (3.76)	3654 (8.17)	9 (6.67)	3657 (8.17)	6 (6.06)
Air temperature levels (°C)										
26-28	19208 (51.33)	3594 (48.26)	20076 (51.08)	2726 (48.99)	22057 (51.04)	745 (45.18)	22741 (50.84)	61 (45.18)	22740 (50.79)	62 (62.63)
More than 28-29	18212 (48.67)	3853 (51.74)	19227 (48.92)	2838 (51.01)	21161 (48.96)	904 (54.82)	21991 (49.16)	74 (54.81)	22028 (49.20)	37 (37.37)
Rainfall levels (mm)										
1000-2000	14820 (39.60)	3505 (47.07)	15817 (40.24)	2508 (45.07)	17450 (40.38)	875 (53.06)	18255 (40.81)	70 (51.85)	18273 (40.82)	52 (52.53)
More than 2000-4000	22600 (60.39)	3942 (52.93)	23486 (59.76)	3056 (54.93)	25768 (59.62)	774 (46.94)	26477 (59.19)	65 (48.15)	26495 (59.18)	47 (47.47)

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Climate information

The average air temperature in PHA 11 was 27.9°C, with a minimum of 27.6°C and a maximum of 28.4°C. The average rainfall was 1,972.7 mm, with a minimum of 1,461 mm and a maximum of 2,319 mm. The average air temperature in PHA 12 was 28°C, with minimum and maximum temperatures of 26.4°C and 29°C, respectively. Its rainfall levels ranged from a minimum of 1,518.4 mm to a maximum of 3,328.3 mm, with an average of 2,253.4 mm (*Table 2*).

Table 2*. The yearly average temperature and total rainfall are presented as classified by the public health areas*

Public health areas	Air temperature (°C)			
	Mean	**SD**	**Min**	**Max**
PHA 11	27.92	0.22	27.60	28.35
PHA 12	28.04	0.61	26.35	28.95
Rainfall (mm)				
PHA 11	1972.66	271.73	1460.95	2319.00
PHA 12	2253.36	434.17	1518.35	3328.25

Testing the association between bacteria contamination and environmental factors

The results from the chi-square test showed that PHA, types of water, years of production, air temperature levels, and rainfall levels were all associated with the detection of TCB and *E. coli*. While PHA, types of water, and years of production were all significantly associated with *S. aureus* and *Salmonella* spp. contamination (*Table 3*).

Table 3. *Associations between bacteria contamination and environmental factors*

Factors	P-value			
	TCB	***E. coli***	***S. aureus***	***Salmonella* spp.**
Public health area	<0.001	<0.001	<0.001	<0.001
Types of water	<0.001	<0.001	<0.001	<0.001
Years of production	<0.001	<0.001	<0.001	<0.001
Air temperature levels	<0.001	<0.001	0.900	0.990
Rainfall levels	<0.001	<0.001	0.090	0.800

Simple logistic regression analysis

Simple logistic regression was used to do an initial assessment of independent variables (bivariate analysis) and discovered that all factors had a likelihood of over the standard for TCB and *E. coli* contamination, whereas factors such as PHA, types of water, and rainfall levels were influenced *S. aureus* contamination. In addition, PHA, types of water, temperature levels, and rainfall levels were caused contaminated *Salmonella* spp.

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Multiple logistic regression analysis

S. aureus contamination exceeding the standard was associated with the types of water. Making ice, consumption water, and processed water which were 17.8, 2.9, and 5.3 times more likely than drinking water to exceed the standard for *S. aureus* contamination, respectively (*Table 4*).

Table 4. *Factors associated with S. aureus bacterial contamination in samples exceeding the standard*

Factors	Crude OR (95%CI)	Adj. OR (95%CI)	P (Wald's test)	P (LR-test)
Types of water				
Drinking	1	1		<0.001
Making ice	16.66 (9.87-28.14)	17.82 (10.48-30.29)	<0.001	
Consumption	4.24 (2.46-7.31)	2.85 (1.52-5.36)	0.001	
Process	2.50 (1.34-4.66)	5.28 (3.01-9.26)	<0.001	

Salmonella spp. contamination exceeding the standard was associated with the PHA, and types of water. The contamination in PHA 11 was 4.34 times more likely to be contaminated with *Salmonella* spp. than that in PHA 12. Making ice, consumption water, and processed water were 2.8, 2.9, and 4.3 times more likely than drinking water to exceed the standard for *Salmonella* spp. contamination, respectively (*Table 5*).

Table 5. *Factors associated with Salmonella spp. bacterial contamination in samples exceeding the standard*

Factors	Adj. OR	95%CI		P (Wald's test)	P (LR-test)
		Lower	Upper		
Public health areas					
PHA 12	1				<0.001
PHA 11	4.34	2.45	7.68	<0.001	
Types of water					
Drinking	1				<0.001
Making ice	2.76	1.35	5.67	0.006	
Consumption	2.87	1.50	5.14	<0.001	
Process	4.30	2.44	7.58	<0.001	

E. coli contamination exceeding the standard was associated with PHA, types of water, years of production, air temperature levels, and rainfall levels. The contamination in PHA 11 was 2.7 times more likely to be contaminated with *E. coli*. Then that in PHA 12. Making ice and processed water were 3.8 and 1.8 times more likely than drinking water to be contaminated with *E. coli*. From 2002 to 2009, the contamination was 11.4, 5.4, 7.6, 4.0, 12.9, 6.5, 5.0, 6.3 times, and from 2011 to 2020, it was 2.1, 2.4, 3.9, 3.4, 5.0, 4.8, 4.3, 8.4, 9.7 and 2.8 times more likely than 2010 to be contaminated with *E. coli*. The air temperature ranged between 28 and 29°C was 1.46 times more likely than

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26-28°C to be contaminated with *E. coli*. More than 2000-4000 mm of the rainfall level was 1.83 times more likely than 1000-2000 mm to exceed the standard for *E. coli* contamination (*Table 6*).

Table 6. *Factors associated with E. coli bacterial found in samples exceeding the standard*

Factors	Adj. OR	95%CI		P (Wald's test)	P (LR-test)
		Lower	Upper		
Public health areas					
PHA 12	1				<0.001
PHA 11	2.70	2.30	3.18	<0.001	
Types of water					
Drinking	1				<0.001
Making ice	3.75	3.25	4.33	<0.001	
Consumption	1.08	0.93	1.25	0.290	
Process	1.75	1.53	2.00	<0.001	
Years of production					
2010	1				<0.001
2002	11.42	5.55	23.48	<0.001	
2003	5.39	2.88	10.10	<0.001	
2004	7.55	3.76	15.14	<0.001	
2005	3.99	2.03	7.86	<0.001	
2006	12.86	5.44	30.43	<0.001	
2007	6.54	3.40	12.61	<0.001	
2008	4.99	2.32	10.74	<0.001	
2009	6.26	3.29	11.93	<0.001	
2011	2.14	1.07	4.27	0.031	
2012	2.43	1.32	4.48	0.004	
2013	3.93	2.17	7.12	<0.001	
2014	3.46	1.70	7.04	<0.001	
2015	4.99	2.66	9.35	<0.001	
2016	4.76	2.62	8.65	<0.001	
2017	4.29	2.34	7.86	<0.001	
2018	8.36	4.50	15.54	<0.001	
2019	9.65	5.21	17.85	<0.001	
2020	2.79	1.56	5.02	<0.001	
2021	1.39	0.74	2.61	0.311	
Air temperature levels (°C)					
26-28	1				<0.001
More than 28-29	1.46	1.16	1.85	0.002	
Rainfall levels (mm)					
1000-2000	1				<0.001
More than 2000-4000	1.83	1.44	2.33	<0.001	

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TCB contamination exceeding the standard was associated with PHA, types of water, years of production, air temperature levels, and rainfall levels. PHA 11 had a 1.4 times greater chance of contamination than PHA 12. Drinking, ice-making, and processing water were 1.8, 2.3, and 1.5 times more likely to be contaminated than water consumption, respectively. From 2002 to 2009, the contamination was higher than 4.5, 3.0, 3.2, 1.9, 4.1, 1.8, 1.5, 3.3 times, the contamination in 2011 was 1.5 times, and the contamination from 2014 to 2020 was 1.5, 1.4, 2.0, 2.2, 2.6 2.7, 2.3 and 1.7 times, which were all more likely than it was in 2021, respectively. The air temperature level at more than 28-29°C was 1.3 times more likely than 26-28°C. More than 2000-4000 mm of the rainfall levels were 1.4 times more likely than 1000-2000 mm to exceed the standard for TCB contamination, respectively (*Table 7*).

Table 7. *Factors associated with TCB found in samples exceeding the standard*

Factors	**Adj. OR**	**95%CI**		**P (Wald's test)**	**P (LR-test)**
		Lower	**Upper**		
Public health areas					
PHA 12	1				<0.001
PHA 11	1.45	1.34	1.57	<0.001	
Types of water					
Consumption	1				<0.001
Drinking	1.8	1.65	1.96	<0.001	
Making ice	2.34	2.1	2.62	<0.001	
Process	1.52	1.38	1.68	<0.001	
Years of production					
2021	1				<0.001
2002	4.54	3.46	5.95	<0.001	
2003	2.97	2.39	3.69	<0.001	
2004	3.17	2.4	4.19	<0.001	
2005	1.84	1.45	2.33	<0.001	
2006	4.07	2.9	5.71	<0.001	
2008	1.53	1.08	2.18	0.017	
2009	3.28	2.66	4.06	<0.001	
2010	1.12	0.87	1.44	0.37	
2011	1.54	1.24	1.91	<0.001	
2012	1.09	0.86	1.38	0.461	
2013	1.08	0.89	1.32	0.423	
2014	1.41	1.12	1.78	0.004	
2015	2.02	1.57	2.6	<0.001	
2016	2.15	1.73	2.67	<0.001	
2017	2.56	2.19	3	<0.001	
2018	2.71	2.27	3.24	<0.001	
2019	2.27	1.77	2.91	<0.001	
2020	1.68	1.39	2.03	<0.001	
Air temperature levels (°C)					
26-28	1				<0.001
More than 28-29	1.32	1.17	1.48	<0.001	
Rainfall levels (mm)					
1000-2000	1				<0.001
More than 2000-4000	1.35	1.19	1.54	<0.001	

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The area under the ROC curve (AUC) was used to evaluate the performance of multiple logistic regression models with the predictive capacity of the fitted model. This demonstrated that the types of water fit the occurrence data well, with 0.82 of AUC in *S. aureus*., and PHA, and types of water fit the occurrence data well with 0.78 of AUC in *Salmonella* spp. In *E. coli* and TCB, PHA, types of water, years of production, temperature levels, and rainfall levels had 0.68 and 0.62 of AUC, respectively.

Discussion

In this study all factors that could impact the water quality. Overall, the study areas, types of water, years of manufacture, air temperature levels, and rainfall levels were associated with statistically significant predictors of TCB and *E. coli* contamination, while the study areas, types of water, and years of production were statistically significant predictors of *S. aureus* and *Salmonella* spp. by chi-square test. Bivariate analysis of independent and dependent variables revealed that all parameters influencing TCB and *E. coli* contamination, while the study areas, water types, rainfall levels affected *S. aureus*, and the study areas, water types, air temperature levels, rainfall levels impacted *Salmonella* spp. Concerning multivariate analysis, the study locations, types of water, years of production, air temperature levels, and rainfall levels were environmental factors influencing the non-standardization of TCB and *E. coli*. In addition, the study locations and types of water influenced *Salmonella* spp. Only *S. aureus* was affected by the types of water.

Based on the findings of this study, the water produced in PHA 11 had higher bacterial contamination than the water produced in PHA 12. The indicator bacteria (TCB) failed the standard more frequently in PHA 12 than in PHA 11, while food poisoning bacteria (*E. coli*, *S. aureus*, *Salmonella* spp.) failed more frequently in PHA 11 than in PHA 12. Food poisoning cases were more common in PHA 11 than in PHA 12, which were corresponded to the previous 5-year morbidity rate data of Thai people’s health statistics at the area level (Department of Diseases Control, 2022). Different areas could produce different types and amounts of bacterial contamination in their water. Similarly, Xu et al. reported in 2019 that the spatial location of the assays affected the climate and bacterial contamination loads (Xu et al., 2019). The study by You et al. in 2019 in China, stated that geographical variations in water resources, environment, topography, and climate affected water quality. The contamination of bacteria in the water was also influenced by weather such as temperature and rainfall, and population density (Poulin et al., 2020).

According to the study, making ice was the most likely to be contaminated with all four types of bacteria, followed by consumption, and drinking water, with statistical significance ($p < 0.05$), indicating that the quality of water that was strictly processed had the potential to detect contamination exceeding the standard. This may be caused by other related factors such as insufficiency of the transportation process, container storage locations, human hygiene during the manufacturing process, types of disinfectants, and filtration efficiency (Badeenezhad et al., 2020). This is similar to a study conducted by Gwimbi et al. (2019) who measured *E. coli* contamination in several bodies of water near the Lesotho Mohale Dam for drinking water production. Besides, there was a study by Onyango et al. (2018) investigating the contamination levels of four bacterial strains, TCB, *E. coli*, *S. aureus*, and *C. perfringens*, in three types of water in Isiolo County, the country: groundwater, surface water, and chlorinated water. Kenya

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also discovered that the number of bacteria in each type of water differed statistically. This study was additionally agreeable with Sriket et al. (2016) investigating TCB contamination in two water sources in Prachinburi Province, eastern Thailand: raw water and tap water. TCB level in raw water was found to be higher than in tap water. According to the Food and Drug Administration requirements, Ministry of Public Health, the presence of TCB, *E. coli*, *Salmonella* spp., and *S. aureus* in the making ice was not detected (Notification of Public Health, 1981, 1984, 2020), which is a more stringent standard than other types of water. This could be another reason why the ice failed to meet the highest standard.

TCB and *E. coli* contamination in water produced in the South Thailand between 2002 and 2021 were significantly different ($p < 0.05$). This contrasted with a study conducted by Onyango et al. (2018) between 2011 and 2016 in Isiolo County, Kenya that no statistically significant difference was found. This variation could be due to climate, seasons, or study periods, resulting in impacts on the levels of bacterial contamination in each water resource (Swistock and Sharps, 2022).

The contamination of TCB and *E. coli* increased as air temperature rose. This is consistent with a 2020 study on fecal coliform bacteria contamination in Uganda and Bangladesh by Poulin et al. (2020). Changes in air temperature were related to changes in water temperature, which affect climate changes and causes changes in water quality in natural water sources. Temperature was considered an important environmental factor because it controlled the maximum amount of dissolved oxygen in water and affects the survival of living organisms (Rajesh and Rehana, 2022). High temperatures contributed to global warming and had negative impacts on the environments (Jung et al., 2014). Similarly, every degree of warming temperature caused by climate changes could influence bacterial growth and survival (Jin, 2016). In fact, indicator bacteria could grow and survive at temperatures ranging from 10 to 45°C (Joklik et al., 1980). Pathogenic bacteria, on the other hand, might begin to die and their concentration might decrease as the temperature dropped (Islam et al., 2017).

Increasing rainfall caused a higher-than-normal detection of bacteria in the water. In this study, TCB and *E. coli* contamination increased as rainfall increased. It is consistent with the studies by Islam et al. (2017) and Poulin et al. (2020) which was found that heavy rains could cause water runoff into bodies of water, and then contaminate them with sediments, nutrients, pollutants, animal waste, and other materials from the community into the water sources, making it unsafe and causing water disease outbreaks (Jung et al., 2014; Poulin et al., 2020). This resulted in waterborne diseases such as diarrhea, cholera, and typhoid fever (Badeenezhad et al., 2020). These outbreaks were linked to pathogen concentrations in the water, and further climate change might increase the risk of waterborne illnesses (Islam et al., 2017).

It could be seen that the study's findings were critical for government agencies and industry to plan and manage the reduction of bacterial contamination in water sources and reach the goal of providing everyone with equal access to clean water. This research also found environmental factors and other water quality had affected the contamination of some bacteria in water sources, such as indicator bacteria (TCB and *E. coli*), which are easily detected. While finding pathogenic bacteria (*S. aureus* and *Salmonella* spp.) in water is difficult due to their short life cycle and ease of destruction from the environmental change (Jin, 2016). Raised consumer safety awareness, and proposed solutions to health problems caused by contaminated water in Southern Thailand. As a result, relevant agencies should inspect every water source to ensure that clean water is

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available to the public. Such studies should be carried out on a regular basis to ensure water quality (Kanno et al., 2020).

The limitations of the present study were the use of the annual report and secondary data, which resulted in incomplete details and could not distinguish the time of manufacture of the samples. Further research is needed on factors such as land use and population density. The goal will be more comprehensive and useful.

Conclusions

Environmental factors, air temperature, precipitation, and other factors include production sites classified by health zones, years of production, and types of water sources were associated with non-standard bacterial contamination of TCB, *E. coli*, *S. aureus*, and *Salmonella* spp. in the South of Thailand. Preventive measures should be taken by the relevant organizations. Water quality must also be checked on a regular basis before it is delivered to consumers, and everyone should have access to clean, safe, easy, and sustainable water.

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Appendix 2

Paper 2: Risk factors affecting the bacterial contamination in water of Thailand's Upper South 2020-2022

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Risk Factors Affecting the Bacterial Contamination in Water of Thailand's Upper South 2020-2022

Kritsanee Ruangsombat[1,2], Apiradee Lim[3], Siriporn Pradit[1,2], Varaporn Cholumpai[4], Prakrit Noppradit[1,2*]

[1] *Marine and Coastal Resources Institute, Faculty Environmental Management, Prince of Songkla University, Hat Yai, Songkhla, Thailand*
[2] *Coastal Oceanography and Climate Change Research Center, Faculty Environmental Management, Prince of Songkla University, Hat Yai, Songkhla, Thailand*
[3] *Department of Mathematics and Computer Science, Faculty Science and Technology, Prince of Songkla University, Rusamilae, Pattani, Thailand*
[4] *Regional Medical Sciences Center 12 Songkhla, Department of Medical Sciences, Ministry of Public Health, Muang, Songkhla, Thailand*

([*]Corresponding author's e-mail: prakrit.n@psu.ac.th)

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Abstract

Bacterial contamination in water is an important cause of human health problems. Water-borne infections are among the top ten reasons for illness in Thailand and accounted for 40 % of all infections necessitating surveillance. The purpose of this study was to identify environmental factors influencing bacterial contamination in water in the upper southern region of Thailand. Secondary data on water quality were obtained from two Regional Medical Sciences Centers, and environmental data were collected from the National Statistical Office's annual reports for 2020 - 2022. A multiple logistic regression model was used to investigate the factors influencing bacterial contamination to exceed the standard. There were 674 water samples contaminated with bacteria, which implied 10.97% of the total number of samples. The factors that were significantly associated with greater bacterial contamination than standard were provinces and years of production, water types, bacterial types, and rainfall levels. Bacteria were more likely to contaminate the water generated in Chumphon province. The quantity contamination for consumption water varied by years of production, with Total Coliform Bacteria (TCB) being the most contaminated bacterial strain as rainfall increases. High precipitation deteriorated biological water quality, which was the origin of water-borne diseases. Entrepreneurs and other connected organizations must constantly watch for bacterial contamination when there is a change in a manufacturing site or when the season changes from hot to rainy.

Keywords: risk factors, bacteria, contamination, water, public health area, Thailand

Introduction

Bacterial contamination in water is a leading cause of human illness. A high bacteria concentration indicates a lack of cleanliness and hygiene [1]. Indicators organisms for biological water quality surveillance can be used to assess cleanliness, and pathogenic bacteria can be used for the detection of water-borne diseases [2, 3]. The presence of an indicator organism indicates the presence of pathogenic organisms [4, 5]. Coliform bacteria (e.g., fecal coliform and *E. coli*) are used as water hygiene indicators since they are not generally detected in clean water [1, 6]. Pathogenic bacteria cause illness when exposed to water, such as *E. coli*, *Salmonella* spp., *Vibrio cholerae*, and *Campylobacter* [7]. Most food and water-borne disease outbreaks are caused by *Shigella* spp., *Vibrio cholerae* [6], *Salmonella* spp., and *E. coli* [8]. Consuming bacterially contaminated water exposes one's health to communicable diseases such as diarrhea, typhoid, dysentery, cholera, and hepatitis A [9]. In addition, waterborne infection epidemics continue to be a major global public health issue. Waterborne diseases can be contracted by eating or drinking contaminated local economy products [7]. Every year, an estimated two billion people drink contaminated water, resulting in an epidemic of 502,000 diarrhea deaths [10], and approximately 3.4 million people die from water-related diseases [11]. Water-borne diarrhea was documented in the 2018

Handbook of Common Diseases in Thailand, which revealed the country's third-highest morbidity rate, with 40% of that infected death [12]. Hepatitis A is also one of the most contagious diseases, followed by hepatitis E and typhoid fever [13]. Disease symptoms might include gastrointestinal illnesses such as severe diarrhea, nausea, jaundice, headaches, and fatigue [4].

Nutrients and environments are the two most principal factors influencing bacterial growth and population [14]. Several studies discovered environmental factors, temperatures, rainfall, and humidity influenced bacterial contamination in water [15 - 19], while others found no relevance but found the types of microorganisms. Geographical locations, seasons, types of water, water treatment process, salinity, organic matters, pH [20], sediment [21], and disease carriers such as flies [5] can be considered all factors that influence water bacterial contamination.

The Upper South of Thailand lies between the Gulf of Thailand to the east and the Andaman Sea to the west. This region is affected by monsoons from the northeast and the southwest, resulting in two seasons: hot and rainy. The average air temperature for the entire year is 20.0 - 28.1°C, the annual accumulated precipitation is 1,418.1 - 4,183.7 mm, and 70 - 83 % relative humidity on average are found [22]. The water quality is acceptable and better than in other regions of Thailand [23]. Bacterial contamination in water can occur at any time during manufacturing, transportation, and storage processes before it reaches consumers [24]. Water quality tests undertaken by government laboratories in the Upper South have repeatedly found that bacterial contamination exceeds the standard level in both raw and processed water [25]. Referring to the standards of Ministry of Public Health Notifications No. 61 and 416, which demand a water quality inspection at least once a year, TCB is less than 2.2, *S. aureus* is fewer than 100 CFU, and *E. coli* and *Salmonella* spp. are not discovered per 100 ml [26, 27]. Additionally, numerous patients during 2020-2022 in the Upper South of Thailand were found to have infections caused by water, with 71,343, 40,200, and 30,877 cases, respectively [28]. It is necessary to look more closely at the environmental factors influencing the prevalence of bacteria in the upper southern region, especially air temperatures and rainfall. Therefore, this study was aimed to identify factors associated with bacterial contamination in water based on the literature review.

Materials and methods

Data source

Water quality data in 2020 - 2022 were obtained from the annual reports of Public Health Area 11 (PHA 11) which consisted of the Regional Medical Sciences Center 11 (RMSC 11) and RMSC 11/1, the Department of Medical Sciences, the Ministry of Public Health. RMSC 11 is in charge of inspecting water quality in four provinces: Chumphon, Ranong, Surat Thani, and Nakhon Sri Thammarat whereas RMSC 11/1 is responsible for inspecting water quality in three provinces: Phang Nga, Phuket, and Krabi. Meteorological data, air temperatures and rainfall in 2020 - 2022 were also taken from the National Statistical Office of Thailand's Statistical Yearbook (**Figure 1**).

Data management

The raw data were acquired from the frequency of water samples tested for bacteriological quality by total sample counts as well as the number of non-standard water samples. That data was classified according to provinces, years of production, water types, and bacterial kinds. The water types were classified into four categories: drinking (clean water for people to drink), ice (water generated by solidification), processed (water used in manufacturing, particularly in the food industry), and consumed (household water such as tap water). The bacterial types were classified into four types: Total Coliforms Bacteria (TCB), *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (S. au), and *Salmonella* spp. (Sal) by counting the total frequency of each type of bacteria for non-standard water quality (or which failed the standard according to the announcement of the Ministry of Public Health No. 61 and 416). The years of production consisted of 3 years: 2020, 2021, and 2022. The provinces consisted of Chumphon, Ranong, Surat Thani, Nakhon Sri Thammarat, Phang Nga, Phuket and Krabi. Air temperatures and rainfall levels were divided into two levels using the average as a criterion. Air temperatures were divided into two levels: less than or equal to 27.5 °C, and more than 27.5 °C. Rainfall was divided into two levels: less than or equal to 2,500 mm, and more than 2,500 mm. The number of water samples that passed and failed inside the standard thresholds was grouped by the provinces, the years of production, the bacterial kinds, the temperature levels, and the rainfall levels.

Statistics analysis

The determinants of this study were environmental elements that were risk factors, specifically the provinces, the years of production, the bacterium types, the water types, the temperature levels, and the rainfall levels as aforementioned. The outcomes of this study were samples that failed to meet the standard criteria, and all were categorical data. The descriptive analysis was performed by statistics of the frequency and the percentage of categorical variables, as well as bacterial prevalence to see the frequency of bacterial contamination in each province, which was calculated having the non-standard samples divided by the total number of the samples and then multiplying by a constant value. Besides, the chi-square test was employed to examine the association between the determinants and the outcomes. The independent variables were then incorporated one by one in a simple model to evaluate their univariate relationships with the results. The independent variables were statistically significant, which were included in the initial multiple logistic regression model. The area under the ROC curve (AUC) was used to evaluate the predictive performance of the model. The results from a model were shown as confidence interval (CI) plots. All statistics and models were analyzed at 95% of the confidence level using the R and RStudio program version 4.1.1 [29].

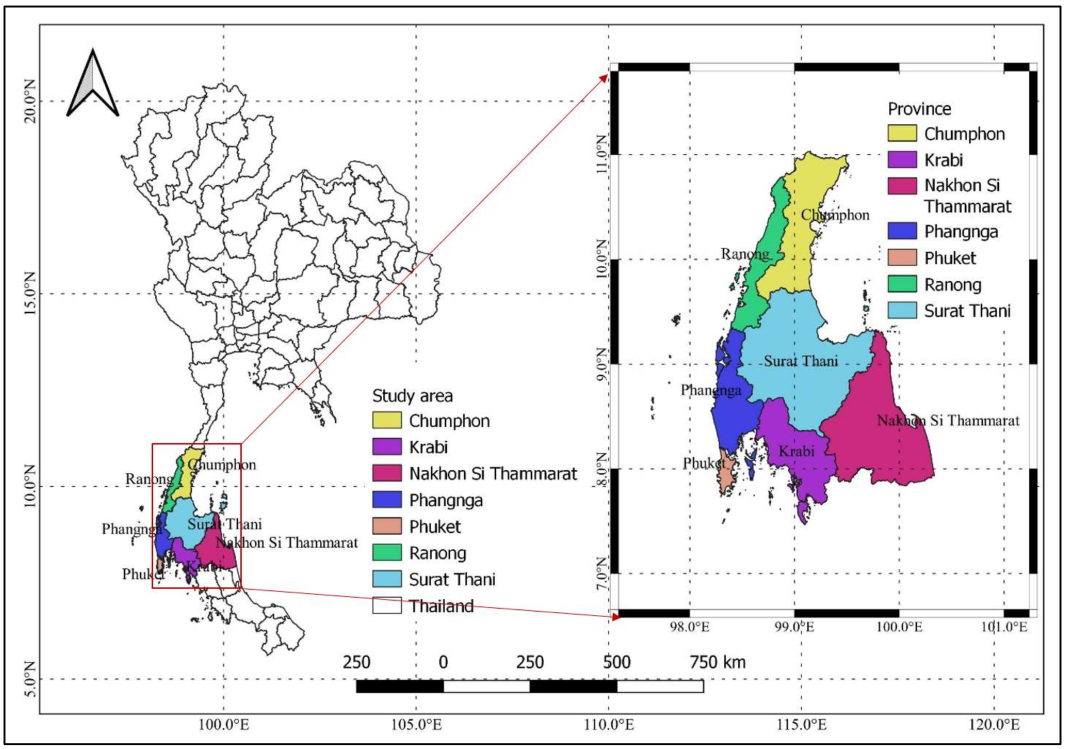

Figure 1 Data sources of 7 provinces in the Upper South of Thailand

Results and discussion

Descriptive analysis of water quality data

In Ranong, the highest prevalence of non-standard water quality was found in 2020, at 21.43 %, whereas the lowest was revealed in Phuket, at 8.37 %. In 2021, the greatest non-standard water was in Ranong at 20 %, while the lowest was in Krabi, at 8.07 %. In 2022, the highest prevalence was shown in Chumphon, at 21.77 %, while the lowest appeared in Phuket, at 7.14 %. In terms of bacteria, the greatest bacterial prevalence was in Ranong (20.9 %), and the lowest was in Phuket (8.6 %) as shown in **Figure 2** (a), (b), (c), and (d). Moreover, an increasing trend of the prevalence of bacteria exceeding the standard was found in Chumphon and Surat Thani, while a decreasing trend of the prevalence of bacteria exceeding the standard was found in Ranong. In Phang Nga, Krabi, Phuket, and Nakhon Sri Thammarat, there were fluctuations in the detection of bacterial contamination. It could be seen that bacterial contamination is common in upper southern Thailand as an effect of the humid tropical, temperatures and topography conducive to bacterial growth and sustenance [23]. From 2017 until the present, all sectors have become conscious of the importance of using water safely. Quality checks are performed on a regular basis. Drinking water, in accordance with Food and Drug Administration regulations [26, 27] and household tap water [30] must meet the required standards. Besides, in accordance with the national strategic plan, the goal is to provide everyone with access to safe drinking water, according to United Nations (UN) agreements to achieve Sustainable Development Goal 6 (SDG 6) [31].

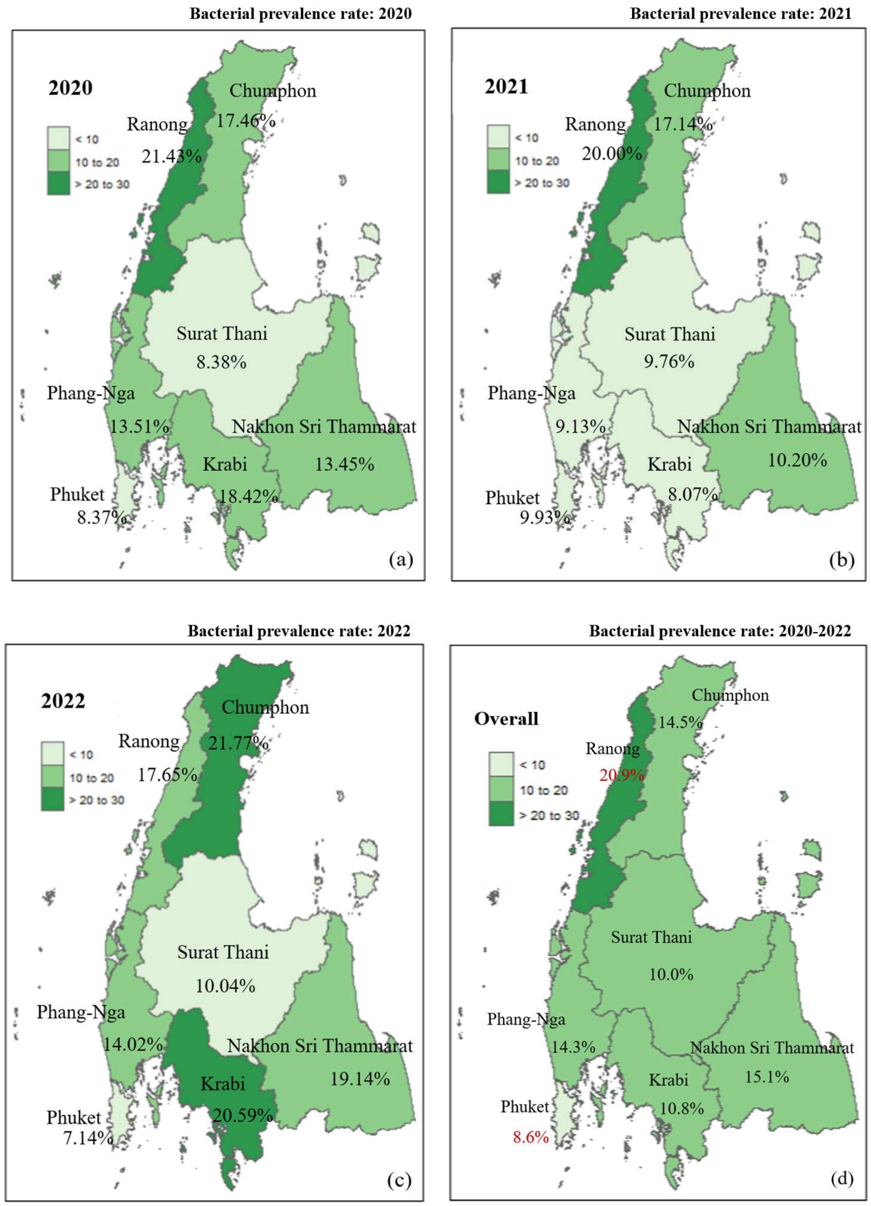

Figure 2 Thematic maps of bacterial prevalence separated into each province and each year of production, in 2020 (a), 2021 (b), 2022 (c), and overall, 2020 - 2022 (d)

Out of the total of 6,142 samples, 674 (10.97 %) exceeded the criteria for bacterial contamination. As shown in **Table 1**, the highest bacterial contamination was found in Ranong (20.87 %), while the lowest was found in Phuket (8.65 %). The prevalence of bacterial contamination exceeding the standard level was the maximum in 2022 (12.25 %) and the lowest prevalence was in 2021 (9.92 %). The consumption water showed the highest level of bacterial contamination (15.44 %) whereas the drinking water was at the lowest level (6.34 %). Importantly, the highest bacterial contamination (12.30 %) was found at air temperatures less than or equal to 27.5 °C, whereas the lowest (0.97 %) was observed at above 27.5 °C. Regarding the rainfall, there was the highest bacterial contamination at greater than 2,500 mm of the rainfall levels (12.64 %). The lowest bacterial contamination was observed at less than or equal to 2,500 mm of rainfall (9.95 %). Furthermore, the highest bacteria contaminated in the water were the TCB strains (24.27 %), while the lowest bacteria were *Salmonella* spp. (0.86 %). In Thailand, agencies under the Ministry of Public Health have measures to monitor water quality to ensure that it is safe for consumption. A handbook on environmental health standards has been produced to guide all sectors in the same direction to manage, prevent, and monitor regularly [32], including a Thailand Drinking Water Standard Guide to ensure that drinking water quality meets quality standards, and strictly enforce laws that contain penalties and prohibit sales [33].

Table 1 Bacterial prevalence rate divided by each risk factors

Risk factors	Total samples	Non-standard samples	Prevalence rate/100
Provinces of production			
Chumphon	365	53	14.52
Ranong	158	33	20.87
Surat Thani	1,484	149	10.04
Nakhon Sri Thammarat	742	122	16.44
Phuket	2,450	212	8.65
Phang Nga	490	56	11.43
Krabi	453	49	10.82
Years of production			
2020	1,530	157	10.26
2021	2,066	205	9.92
2022	2,546	312	12.25
Water types			
Drinking	2,635	167	6.34
Ice	915	109	11.91
Processed	169	24	14.20
Consumption	2,423	374	15.44
Bacterial types			
TCB	1,949	473	24.27
E. coli	1,625	161	9.91
S. aureus	1,274	29	2.28
Salmonella spp.	1,294	11	0.86
Rainfall levels (mm)			
≤ 2,500	3,808	379	9.95
> 2,500	2,334	295	12.64
Air temperature levels (°C)			
≤ 27.5	2,960	364	54.01
> 27.5	3,182	310	45.99

Descriptions of meteorological data

The average air temperature in Thailand's Upper South in 2020 - 2022 was 27.70 ± 0.19 °C. The highest average temperature was at 29.03 ± 0.25 °C in Phuket, while the lowest was at 27.27 ± 0.23 °C in Surat Thani. The average annual rainfall was 2,573.33 ± 526.99 mm. The highest rainfall amount was measured in Ranong, with 4,133.47 ± 1,271.74 mm, while the lowest rainfall was recorded in Surat Thani, with 1,562.63 ± 438.41 mm, as stated in **Table 2**. This information is congruent with statistics from the Regional Environment Office 15, which shows that the highest total rainfall appears in Ranong and Phang Nga, known as the eight-month rainy city with only four-month sunshine. In addition, in Thailand's upper southern area, the highest average air temperature is in Phuket because its most locations are mountainous plains, and it receives monsoon winds all year [23].

Table 2: The average and standard deviation of meteorological data divided by provinces between 2020 and 2022

Provinces	Average ± SD	
	Air temperatures (°C)	Rainfall (mm)
Surat Thani	27.27 ± 0.23	1562.63 ± 438.41
Chumphon	27.50 ± 0.17	1864.87 ± 170.65
Nakhon Sri Thammarat	27.63 ± 0.15	2663.77 ± 507.86
Ranong	27.60 ± 0.26	4133.47 ± 1271.74
Phuket	29.03 ± 0.25	2068.87 ± 449.48
Krabi	27.30 ± 0.17	1850.70 ± 398.28
Phang Nga	27.53 ± 0.12	3868.27 ± 452.51
Average	27.70 ± 0.19	2573.33 ± 526.99

The results of the chi-square test and the univariate analysis revealed that bacterial contamination above the standard were substantially associated with the provinces of production, the water types, the production years, the bacterial types, the temperature levels, and the rainfall levels (explained in supplementary file S1). **Table 3**, showing the results of the multiple logistic regression model, revealed that the provinces of production, the water types, the production years, the bacterial types, and the precipitation levels increased the risk of detecting bacterial contamination above the standard. The performance of the model, which was evaluated by the ROC curve, showed high accuracy (AUC = 0.82) and good convergence with the actual data.

The model was able to predict the likelihood of finding bacterial contamination in Surat Thani, Chumphon, Nakhon Sri Thammarat, Ranong, and Krabi, where there was greater bacterial contamination than the standard, with 1.93, 3.35, 2.56, 2.28, and 3.91 times, respectively, when compared to Phuket. This study found that bacterial contamination varied according to the producing provinces. Most of the southern province areas are plateaus with large mountain ranges and coastal plains. When a monsoon breeze blows through, there is a lot of rain, and the weather varies throughout the year. Due to the various geographic and climatic variables like temperatures, precipitations, sediments, turbidity, and retention periods, the types and degrees of bacterial contamination were influenced [34]. Similarly, You et al.'s 2019 study discovered that variations in geography, environments, terrains, and climates caused changes in water quality [35]. In 2006, Thanphuphasiam et al. evaluated the difference in water quality between the tsunami-affected area and the nearby untouched areas. The two regions were discovered to have varied water quality [36]. Similarly, Bastaraud et al. stressed that the main factor of bacterial contamination was the location of the production facility [37]. This also aligns with the research conducted by Archer et al., which indicated that climate change and geography contributed to the increased spread of bacteria [30].

According to the water types, when compared to the drinking water, the bacteria contamination in the ice, the process water, and the consumption water were found to be 2.4, 2.79, and 3.41 times higher, respectively. The consumption water or the tap water was more prone to bacterial contamination than the regular drinking, the ice, and the process water. Tap water was mostly derived from well water, underground water, and rain. Chlorine and sand filters were used to eliminate microorganisms and stored in big containers before being piped to households [38]. Cross-contamination can occur during transmission to homes over damaged pipes. Unclean containers and prolonged storage can increase the number of bacteria growths as well [39]. The current study was additionally consistent with the study of Thongkhao et al.'s 2021 conducted in Nakhon Sri Thammarat, Thailand, which discovered non-standard water quality due to a lack of regular quality control, resulting in a major cause of bacterial contamination [40]. In fact, water which is processed, such as drinking water, ice, and food processing water, is required to store in non-recyclable materials and should contain low bacterial contamination; therefore, water producers must be fined, banned, and halted for sales, and production until the water quality meet this criterion standard [41].

The bacterial contamination was 1.45 times higher in 2022 than in 2021. The number and types of bacteria could be identified depending on their life cycles, living habitats, and the periods of the study time [6]. This study indicated that the quantity of bacterial contamination in water fluctuated and exceeded the highest standard in 2022. This could be due to environmental changes that caused the overall rainfall in

2022 to be higher than in 2020-2021 [22]. This was considered one of the reasons for the increased bacterial contamination. These results were congruent with the findings of Poulin et al., who discovered that strong rains could carry sewage from the neighborhood or agricultural runoff into water sources; bacterial contamination, therefore, rises. [42].

Types of bacteria were associated with water quality that did not meet the specified standards. For each bacteria type, *S. aureus*, *E. coli*, and TCB were 2.79, 13.46, and 40.47 times more contaminated than the norm, respectively, when compared to *Salmonella* spp. This study discovered that the indicator bacteria (TCB and *E. coli*) were more likely found than the pathogenic bacteria (*S. aureus* and *Salmonella* spp.) to surpass the standard criteria. The presence of these bacteria indicated that there was a reasonable chance of detecting pathogenic bacteria in the water [43]. This finding was consistent with Yates' 2019 study, which discovered that the indicator bacteria were always identified before the pathogenic bacteria were detected, and they were typically more difficult to find in nature and grow in the environment than TCB and *E. coli* [21].

In addition, rainfall also influenced bacteria counts and water quality. When the rainfall amounts exceeded 2,500 mm, bacterial contamination was 1.78 times higher than it was with less than or equal to 2,500 mm of the rainfall amounts. This analysis revealed that when the rainfall amounts increased, there was an increase in bacterial contaminants in the water. Similarly, a 2019 study on factors impacting on water bacterial pollution by Seo et al. in South Korea discovered that higher rainfall increased the rate of bacterial contamination [44]. Likewise, Julie et al. discovered in a 2012 - 2014 study that higher rainfall in Kenya caused changes in water quality and increased bacterial contamination, as sewage from houses and farmland infiltrated the water supply [45]. In addition, an investigation conducted by Love and Laub in Texas found substantial evidence linking increased rainfall to a higher concentration of bacteria. Their study, conducted in both urban and rural areas, emphasized that this correlation was particularly pronounced within city environments [46].

Table 3 Factors association with the bacterial contamination in the Upper South of Thailand

Risk factors	Crude OR (95 % CI)	Adj OR (95 % CI)	*p - value*
Provinces of production			< 0.001
Surat Thani	1.18 (0.95, 1.47)	1.93 (1.46, 2.56)	
Chumphon	1.79 (1.30, 2.48)	3.35 (2.27, 4.93)	
Nakhon Sri Thammarat	2.08 (1.63, 2.64)	2.56 (1.85, 3.54)	
Ranong	2.79 (1.85, 4.19)	2.28 (1.36, 3.80)	
Phuket	1.00	1.00	
Krabi	1.28 (0.92, 1.78)	2.91 (1.91, 4.43)	
Phang Nga	1.36 (1.00, 1.86)	1.46 (0.98, 2.16)	
Water types			< 0.001
Drinking	1.00	1.00	
Ice	2.00 (1.55, 2.58)	2.40 (1.81, 3.20)	
Process	2.45 (1.54, 3.87)	2.79 (1.68, 4.63)	
Consumption	2.70 (2.23, 3.27)	3.41 (2.75, 4.23)	
Years of production			< 0.001
2020	1.04 (0.83, 1.29)	1.19 (0.83, 1.71)	
2021	1.00	1.00	
2022	1.27 (1.05, 1.53)	1.45 (1.09, 1.94)	
Bacterial types			< 0.001
Salmonella spp.	1.00	1.00	
Staphylococcus aureus	2.72 (1.35, 5.46)	2.79 (1.38, 5.61)	
Escherichia coli	12.83 (6.93, 23.73)	13.46 (7.26, 24.98)	
Total Coliforms Bacteria	37.38 (20.46, 68.27)	40.47 (22.09, 74.13)	
Rainfall levels (mm)			< 0.001
≤ 2,500	1.00	1.00	
> 2,500	1.31 (1.11, 1.54)	1.78 (1.27, 2.50)	
Temperature levels (°C)			< 0.001
≤ 27.5	1.00	1.00	
> 27.5	0.77 (0.66, 0.90)	0.91 (0.58, 1.42)	

Remarks: Crude OR (crude odds ratio) = cOR, Adj OR (adjust odds ratio) = aOR

This study discovered that the provinces, the years of production, the types of water, the bacterial species, and the rainfall levels were the major risk factors for above-standard bacterial contamination in the water in Thailand's Upper South. The likelihood of bacterial contamination was the greatest in Chumphon, particularly in 2022. TCB was the most prevalent in the consumption water and rose with the increasing rainfall. Furthermore, this information can act as a database for all relevant organizations to conduct holistic water management, fostering collaboration to provide clean, safe, and sufficient water quality for everybody. Sustainable access is consistent with the United Nations National Strategic Plan and SDG 6 [31]. A 95 % confidence intervals plot of bacterial prevalence rate was split by the overall mean at 10.97 % (red line) as shown in **Figure 3**. In RMSC 11, there was more bacterial prevalence than in RMSC 11/1. That is, the highest prevalence of the bacteria was found in Chumphon, followed by Nakhon Sri Thammarat and Ranong, respectively. The prevalence of these three provinces was also higher than the overall average, indicating a high risk of the bacterial contamination exceeding the standard. The results also showed the prevalence of the bacteria exceeding the highest standard in 2022 in the consumption water, with TCB bacteria being the most prevalent when the rainfall amount was greater than 2,500 mm. According to the study's findings, the issues to be researched are environmental risk factors that influence water pollution when it changes. This is because the volume and kinds of bacteria vary depending on their habitats and environments [47]. As a result, reducing the risk of bacterial contamination requires regulating environmental changes and being cautious at every stage of the process, from production, transportation to customers [48].

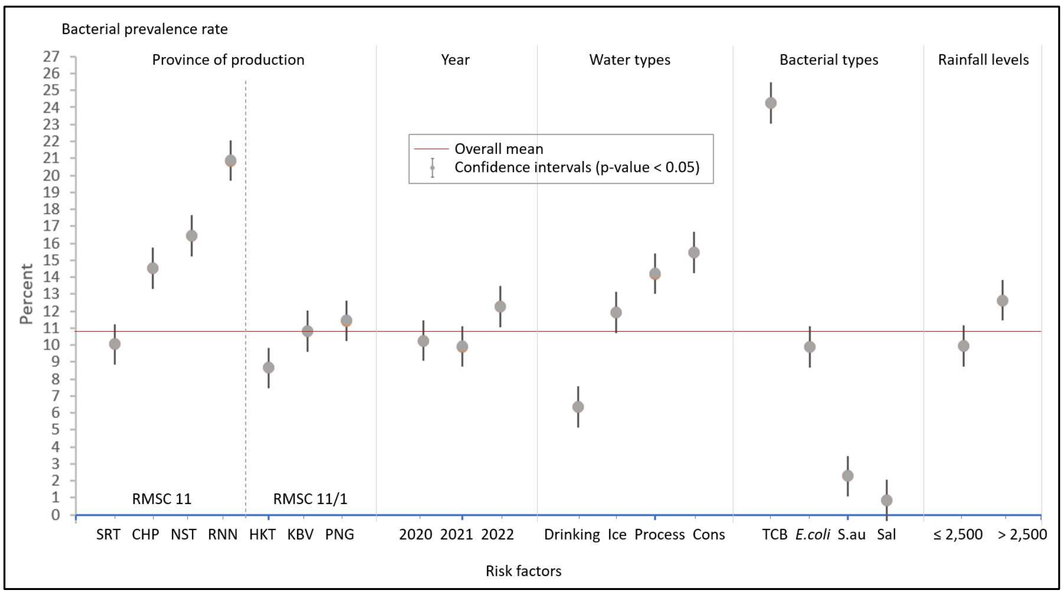

Figure 3 The 95 % CI of the factors influencing the bacterial prevalence in the water from the multiple logistic regression model
Note: SRT is Surat Thani, CHP is Chumphon, NST is Nakhon Sri Thammarat, RNN is Ranong, HKT is Phuket, KBV is Karabi, PNG is Phang Nga, and Cons is Consumption.

Study limitations

This investigation of the secondary data was employed in this study. As a result, there were some limitations on the details that can be accessed. There was also the possibility of bias or misunderstanding in the data analysis. In addition, this was the initial study of the environmental factors affecting bacterial contamination in water. Therefore, the information and the duration were restricted. Further research should be conducted by expanding the research duration, adding spatial data, and physical and chemical water quality, all of which could influence bacterial contamination.

Conclusions

This study demonstrated that the most significant environmental factors affecting bacterial contamination in water exceeding the required levels in Thailand's upper southern region were the locations and the years of production, the types of water, the types of bacteria, and the rainfall amounts. These were also the main contributors underlying rising water-bacterial contamination, which caused infections transmitted through the water. Bacteria contamination in water could become a public health issue in Thailand. Monitoring changes in risk variables such as greater precipitation during the rainy season or modifications in the locations of water production are critical that operators and relevant authorities should rigorously supervise.

Acknowledgments

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Appendix 3

Proceeding 1: Relationship between environmental factors influencing the detection of total coliforms bacteria in drinking water in sealed containers, Southern Thailand by multiple logistic regression.

HOLISTIC HEALTH AND WELLNESS MANAGEMENT

Relationship between Environmental Factors Influencing the Detection of Total Coliforms Bacteria in Drinking Water in Sealed Containers, Southern Thailand by Multiple Logistic Regression.

Kritsanee Ruangsombat[1] and Prakrit Noppradit[2]

Faculty of Environmental Management
Prince of Songkla University
Songkhla, Thailand
Email: ruangsombat@hotmail.com[1]
Email: prakrit.n@psu.ac.th[2]

Abstract
The safety of drinking water is essential to human life. However, previously, the numbers of bacterial quality of drinking water in sealed container was beneath designated safety standards. Although drinking water quality in Thailand is controlled according to the Notification of the Ministry of the Public Health No. 61 (BE. 2524) entitled Drinking water in sealed containers. Therefore, this study applied statistics to determine the relationship between the environmental factors, the growth and proliferation of bacteria. The analyzed annual reports of water quality (high total coliforms bacteria sample) by the Department of Medical Science laboratories were obtained, parallelly the reported annual air temperature was collected via National Statistical Office during fiscal year 2015 – 2020. The bacterial water quality indicator, here expressed by the high total coliforms bacteria (TCB) sample in drinking water in sealed container, was applied to multiple logistic regression by setting the level of confidence 95% ($p < 0.05$). The result showed that year of production and each laboratory have been significantly difference. This may be caused by different climate conditions, especially temperature, which is an important factor influencing the growth of bacteria. Moreover, the growth of the city, transportation process and location for keep might affect the water quality and further influence drinking water quality. However, process of manufacture does not ensure the pass of product testing, further study from production to distribution processes of sealed-container drinking water should be done.

Keywords: total coliform bacteria, drinking water, southern Thailand, multiple logistic regression

1. Introduction

Water is essential for human required in life (Usuwanthim *et al*, 2010, 50-56). Which is a basic facility giving everyone access to clean, safe, and affordable water. It is the United Nationals (UN) declared the Sustainable Development Goals 6 or SDG 6 with the aim clean water and sanitary for all by 2030 around the world (Alshomali & Gulseven, 2020, 1-3). Human need average for clean water in residence about 100 liters/person/day (Office of the Health Promotion Fund., 2012). Water quality is important factors affecting health safety of consumer and the suitability for utilization in various aspects (Bajarczuk *et al*, 2018, 10102-10114). Clean water is to be use for drinking, cooking, and simple hygiene (Khalifa & Bidisee, 2018, 17-20). Presently, around the world required use water increase 1 percent/year and expect the human would like to use increase about 20 – 30 times of consumption within 2090 (Singhanart, 2020). During 2010 – 2017, Demand of drinking water was increase from 61 to 71 percent (Poulin *et al*, 2020, 13566-13578). Natural resource of water was appropriated for manufacture water supply was surface water, such as, rain, creeks, rive, lakes and can used another there but bring to improve water quality before (Thongboran & Srisuwan, 2018, Khalifa & Bidaisee, 2018, 17-20, Stanaszek-Tomal, 2020, 1-9). In 2005 – 2014, Bureau of water quality management analyze surface water can showed more deteriorating water quality by main caused discharge of sewage from community sources and leaching of agricultural residues. Regarding water quality by region, Southern Thailand have been the best than another region. Overall was on fair to good criteria (Bureau of water Quality Management, 2014). Consider, provinces each found that, Phuket was the most degraded, Phang-nga was faired, Krabi Trang and Ranong were good in standard (Regional Environment Office 15, 2019). Chumphon was fair to good, Nakhon Si Thammarat is degraded to faired, Surat Thani and Phatthalung were degraded to good (Regional Environment Office 14, 2021). The continuous development of tourism, agriculture and industry cause water pollution and water quality were more deteriorate (Islam *et al*, 2017, 319-332). Water quality assessment can be defined as the evaluation of the biological, chemical, and physical (Bajarczuk *et al,* 2018, 10102-10114, Jufri, 2020, 12-17). Bacterial indicators of water quality, total coliforms bacteria (TCB) can be easy to analyze more than the other because pathogens have short life cycle, low quantity and difficult for analyze (Seo *et al*, 2019, 1-16). They live intestinal gut and can be detected in digestive system of warm-blood animals including human, if detected in water resource it indicates excretion feces contamination (Nuangjui & Chanphiwat, 2021, 11-16, Seo *et al*, 2019, 1-16). In addition, TCB can cause serious illness such as gastroenteritis and diarrhea, these contaminations can indication with pathogens with cause diseases in the human such as cholera, dysentery, typhoid fever from infection *Vibrio cholerae*, *Shigella dysenteries* and *Salmonella typhimurium* (Kudsong *et al,* 2010, 102-106, Nimrat *et al*, 2021, 102-113, Takal & Quaye-Ballard, 2018, 1-11). The quality standard TCB per 100 milliliter must less than 2.2 in drinking water in sealed container (Notification of Public Health No. 61, 1981) and product samples must analyze at least once per year.

From the data previous, in 2010 found cause TCB contamination in the base-basin of the Nan River exceeds the specified standard, it was illness from incidents in water in Thailand, which make must be vigilant and notified of inappropriateness for consumption (Kudsong *et al,* 2010, 102-106). Later in 2015, There was a study showed have been TCB does not pass specified standard from consumption behavior in the river of the people in Bang Look Sua Sub-District community, Ongkharak District, Nakhon Nayok Province, while testing relationship statistic significant between occurrence of diarrhea and skin disease (Phakham *et al,* 2016, 17-23). At the same time, 1,330 staffs and students detected 55 people were diarrhea epidermic at military school from Prachuap Khiri Khan Province because of drinking water contaminated *Escherichia coli* (Komol *et al*, 2018, 124-135). For overseas situation, between 2010 – 2017 the population was about 2.2 million people have been diarrhea due to drinking water contamination with microorganism and 500,000 deaths in Republic of Uganda and Bangladesh (Poulin *et al*, 2020, 13566-13578). The drinking water quality each area found that was difference and had increase deterioration. In this study applied statistical analysis to determine the relationship between the environmental factors and the not pass the standard of microorganism. The bacterial water quality indicator, here expressed by the high TCB sample in sealed-container drinking water, was applied to multiple logistic regression model, and predict the likelihood (Aungsakaw & Jhan-o-phat, 2016).

2. Research Objectives

This study aimed to determine the relationship between environmental factors and the high TCB sample in drinking water in sealed containers Southern Thailand.

3. Research Methodology

3.1 The annual reported high TCB sample in drinking water in sealed container was collected from the Department of Medical Science laboratory in Southern Thailand. Data consisted of four laboratories based on Regional Medical Science Center (RMSC) setting; Surat Thani = RMSC 11 (responding for Surat Thani, Nakorn Sri Thammarat, Chumphon, and Ranong Provinces); Phuket = RMSC 11/1 (responding for Phuket, Phang Nga and Krabi Provinces); Songkhla = RMSC 12 (responding for Songkhla, Pattani, Yala, and Narathiwat Provinces) and Trang = RMSC 12/1 (responding for Trang, Satun and Pattalung Provinces). Then, the reported TCB were further normalized by the number of the total samples. Moreover, the annual air temperature data was collected from National Statistical Office (NSO) between fiscal year 2015 and 2020. The air temperature was averaged based on the regional laboratory responsibility. In Figure 1.

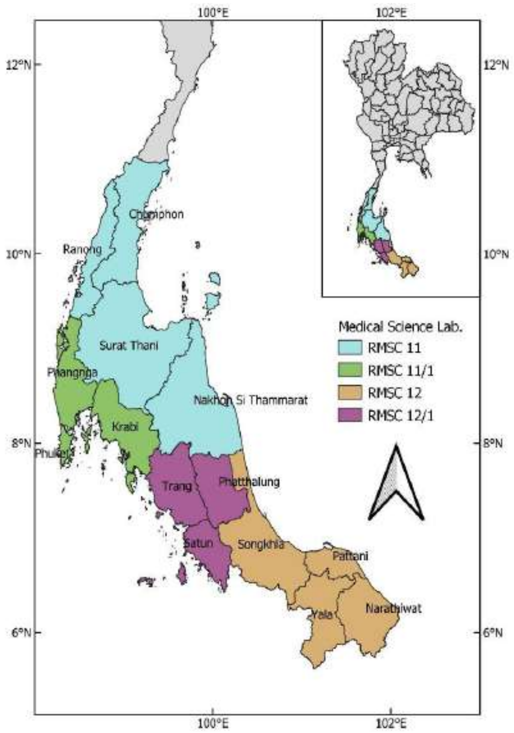

Figure 1 Four Regional Medical Science Center laboratories in Southern Thailand;
blue: RMSC 11 (Surat Thani Laboratory), green: RMSC 11/1 (Phuket Laboratory), purple: RMSC 12/1 (Trang Laboratory), and brown: RMSC12 (Songkhla Laboratory).

3.2 To determine the relationship of air temperature factors and high TCB samples in sealed-container drinking water, collected data was analyzed with the multiple logistic regression model with the 'stats' package in R 4.1.1 (Lim, 2021, Raksakiattisak, 2018). The number of the high TCB sample was used as a dependent variable, and environmental factors (year of production, air temperature, and RMSC laboratory) are predictive variables.

3.3 A multiple logistic regression model is in the follow form the odds or the logit (y), logit (y) = $\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3$ when y is number of high TCB sample, x_1 is year of production, x_2 is air temperature, x_3 is RMSC laboratory. Test probabilistic ratio (Likelihood ratio) by Analysis of variance (ANOVA) test for p = 0.95 were used to determine the significance of differences in the examined parameters between the study area of the year. Then, the no significant variables were eliminated. Finally, the computed model was rechecked the suitability of the model with the ROC curve (Lim, 2021).

4. Results

Table 1 presents the collecting quality report about total sample of drinking water, high TCB sample all Regional Medical Sciences Center laboratory (RMSC 11 = Surat Thani = SRT, RMSC 11/1 = Phuket = PKT, RMSC 12 = Songkhla = SKL, RMSC 12/1 = Trang= TRG) and average air temperature from National Statistical Office (NSO) between 2015 - 2020. The water samples from 4 laboratories were total 10,665 samples (consist of 3,932 samples in RMSC 11, 1,070 samples in RMSC 11/1, 2,856 samples in RMSC 12, 2,807 samples in RMSC 12/1), high TCB sample was 1,795 samples (consist of 922 samples in RMSC 11, 241 samples in RMSC 11/1, 314 samples in RMSC 12, 318 samples in RMSC 12/1) and average air temperature range from 27.48 to 27.83°C (consist of 27.48°C in RMSC 11, 27.86C in RMSC 11/1, 27.70C in RMSC 12 and 27.83C in RMSC 12/1).

Table 1 Quality report on drinking water and air temperature form RMSC laboratory and NSO

Year	Number of samples								Mean of air Temp (°C)			
	RMSC 11		RMSC 11/1		RMSC 12		RMSC 12/1		RMSC 11	RMSC 11/1	RMSC 12	RMSC 12/1
	Total	High TBC	Total	High TBC	Total	High TBC	Total	High TBC				
2015	880	298	222	34	532	23	464	52	27.45	27.83	28.03	27.83
2016	906	164	166	28	707	159	465	52	27.70	27.97	28.15	28.30
2017	615	115	143	42	555	53	422	42	27.25	27.53	27.28	27.57
2018	580	130	256	42	372	32	799	73	27.28	27.73	27.38	27.63
2019	530	137	172	48	343	11	290	39	27.63	28.10	27.83	27.90
2020	421	78	111	47	347	36	367	60	27.60	27.97	27.50	27.77
Total	3,932	922	1,070	241	2,856	314	2,807	318	27.48	27.86	27.70	27.83

Table 2 presents the results of analysis multiple logistic regression revealed that the relationship between year of production (x_1) and air temperature (x_2). The p-values were ranged from 7.52 x 10^{-8} to 0.049 (p < 0.05). A logistic regression model was created to predict the likelihood of high TCB sample separate each laboratory showed that

(1) $\text{logit}(y)_{\text{RMSC 11}} = 262.06 - 0.10x_1$
(2) $\text{logit}(y)_{\text{RMSC 11/1}} = -659.65 + 0.26x_1$
(3) $\text{logit}(y)_{\text{RMSC 12}} = 913.97 - 0.35x_1 - 0.56x_2$
(4) $\text{logit}(y)_{\text{RMSC 12/1}} = -202.12 + 0.07x_1 + 0.56x_2$

Analysis of variance (ANOVA) showed that environmental factors in year of production, air temperature and the high TCB sample of drinking water in sealed containers at Southern Thailand, there were statistically significant differences between each laboratory.

Table 2 The result of statistic test classified by each laboratory.

RMSC 11	Estimate Std.	Error	z value	Pr (>lzl)
(Intercept)	262.06	64.39	4.07	4.71×10^{-5} ***
Year (x_1)	-0.10	0.02	-4.12	3.77×10^{-5} ***
Temp (x_2)	-0.23	0.21	-1.07	0.284
RMSC 11/1	**Estimate Std.**	**Error**	**z value**	**Pr (>lzl)**
(Intercept)	-659.65	141.59	-4.66	3.18×10^{-6} ***
Year (x_1)	0.26	0.06	4.54	5.64×10^{-6} ***
Temp (x_2)	-0.43	0.37	-1.14	0.254
RMSC 12	**Estimate Std.**	**Error**	**z value**	**Pr (>lzl)**
(Intercept)	913.62	169.86	5.38	7.42×10^{-8} ***
Year (x_1)	-0.35	0.06	-5.38	7.52×10^{-8} ***
Temp (x_2)	-0.56	0.11	-4.93	8.25×10^{-7} ***
RMSC 12/1	**Estimate Std.**	**Error**	**z value**	**Pr (>lzl)**
(Intercept)	-202.12	94.21	-2.14	0.032 *
Year (x_1)	0.07	0.04	1.97	0.049 *
Temp (x_2)	0.56	0.27	2.07	0.039 *

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 3 presents overall the relationship between year of production (x_1), air temperature (x_2) and RMSC laboratory (x_3) were differences in the detection number of analyze high TCB sample in sealed containers drinking water Southern Thailand. A multiple logistic regression model showed that

$$\text{logit}(y) = -2.03 - 0.27x_{1(2560)} - 0.32x_{1(2561)} + 0.03x_2 - 0.87x_{3(\text{RMSC } 12)} - 0.81x_{3(\text{RMSC } 12/1)}$$

Year of production, RMSC laboratory were significant differences. The p-values were ranged from $< 2 \times 10^{-16}$ to 0.03 ($p < 0.05$) and air temperature was not significant differences ($p > 0.05$) in high TCB sample between year of production and RMSC laboratory.

Table 3 The statistic result and multiple logistic regression model Southern Thailand.

	Estimate Std.	Error	z value	Pr (>lzl)
(Intercept)	-2.03	6.97	-0.29	0.771
Year (x_1) 2559	-0.06	0.10	-0.63	0.529
Year (x_1) 2560	-0.27	0.12	-2.21	0.027 *
Year (x_1) 2561	-0.32	0.11	-3.00	0.003 **
Year (x_1) 2562	-0.12	0.09	-1.31	0.190
Year (x_1) 2563	-0.03	0.09	-0.29	0.772
Temp (x_2)	0.03	0.25	0.13	0.893
Lab(x_3) RMSC 12	-0.87	0.09	-8.76	$< 2 \times 10^{-16}$ ***
Lab (x_3) RMSC 11	0.04	0.13	0.34	0.730
Lab (x_3) RMSC 12/1	-0.81	0.09	-8.56	$< 2 \times 10^{-16}$ ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 4 presents the suitability examining of the environmental factor variables present or absence in the multiple logistic regression models by statistics chi-square (χ^2- test). The result showed all environmental factors are p-value < 0.05. Which can conclusion the year of production, air temperature and RMSC laboratory must have into the model.

Table 4 Testing the suitability of variables in logistic regression equation by χ^2 - test

Factor	Df Deviance	Resid.	Df Resid.	Dev	Pr (>Chi)
NULL			23	557.51	
Year (x_1)	5	33.51	18	524.00	2.98×10^{-6} ***
Temp (x_2)	1	95.92	17	428.08	2.2×10^{-16} ***
Lab (x_3)	3	173.19	14	254.89	2.2×10^{-16} ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Based on figure 2, the equation can predict the probability the detection of TCB sample by $p(y) = e^{\text{logit}(y)} / 1 + e^{\text{logit}(y)}$, when $p(y) < 0.5$ is the low TCB sample and $p(y) \geq 0.5$ is the high TCB sample. After that perform an evaluation of the model's performance by recheck the harmony with the ROC curve which considers sensitivity and specificity area under the curve along with comparing result and true value from observation, the ROC curve = 0.63. It shows that the model's poor alignment with the actual data has a chance of predicting the result incorrectly.

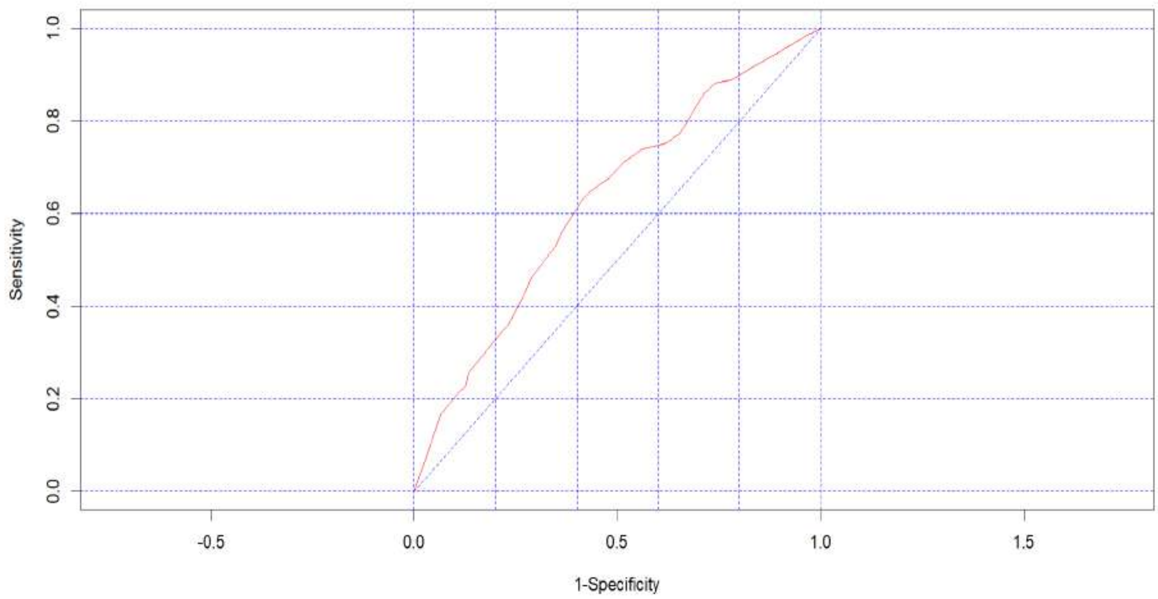

Figure 2 The testing harmony of the model with ROC curve.

Table 5 presents the suitability testing process of the generated model was concluded, the likelihood of high TCB sample in drinking water in sealed container have relationship with year of production, air temperature and RMSC laboratory. Their showed that, which comparing the number of high TCB sample in 2015 with 2017 and 2018, they dropped out 0.76 and 0.73 times higher respectively. And comparing the testing sites with each laboratory with RMSC 11/1, it was found that the high TCB sample of the RMSC 12, and RMSC 12/1 were 0.42 and 0.44 times respectively.

Table 5 The evaluated result from models created with multiple logistic regression.

	Crude OR (95%CI)	Adj. OR (95%CI)	P (Wald's test)	P (LR-test)
Year: ref. = 2015				< 0.001
2016	0.91 (0.78, 1.06)	0.94 (0.77, 1.15)	0.529	
2017	0.71 (0.59, 0.84)	0.76 (0.6, 0.97)	0.027	
2018	0.67 (0.56, 0.79)	0.73 (0.59, 0.9)	0.030	
2019	0.89 (0.74, 1.06)	0.88 (0.73, 1.06)	0.190	
2020	0.9 (0.75, 1.07)	0.97 (0.81, 1.17)	0.772	
Temp	0.72 (0.6, 0.86)	1.03 (0.63, 1.69)	0.893	< 0.001
Lab: ref. = RMSC 11/1				< 0.001
RMSC 12	0.42 (0.35, 0.521)	0.42 (0.34, 0.51)	< 0.001	
RMSC 12/1	0.44 (0.37, 0.53)	0.44 (0.37, 0.54)	< 0.001	
RMSC 11	1.05 (0.9, 1.24)	1.04 (0.81, 1.34)	0.773	

5. Discussion and Conclusion

The aim of this study was investigated detection high TCB sample in drinking water in sealed containers under the 3 environmental factors, (1) year of production (2) air temperature and (3) RMSC laboratory. We found that all environmental factors have been relationship statistic significant ($p_{(x1)}$, $p_{(x2)}$ and $p_{(x3)} < 0.05$). The relationship separates each laboratory, the result show that, year of production in RMSC 11 and RMSC 11/1 have been relationship statistic significant ($p_{(x1)\ (RMSC\ 11)}$ and $p_{(x1)\ (RMSC\ 11/1)} < 0.05$) and air temperature are not statistic significant ($p_{(x2)\ (RMSC\ 11)} > 0.05$ and $p_{(x2)\ (RMSC\ 11/1)} > 0.05$). At the same time, RMSC 12 and RMSC 12/1 are statistic significant ($p_{(x1)\ (RMSC\ 12)}$, $p_{(x2)\ (RMSC\ 12)} < 0.05$ and $p_{(x1)\ (RMSC\ 12/1)}$, $p_{(x2)\ (RMSC\ 12/1)} < 0.05$).

Because of difference year and location of production are influencing to climate change, such as, temperature which influence to growth and survival of all bacteria (Hou *et al*, 2017, 1-12, Islam *et al,* 2017, 319-332). The air temperature each location has been difference, while was suitable for growth of bacteria between 20 – 50 degrees Celsius (Bintsis, 2017, 529-563). The detection high TCB in water does not pose a direct risk of waterborne diseases, but their indicates contamination and the possible presence of waterborne pathogens which risk of waterborne disease outbreaks to the consumer (Bojarczuk *et al,* 2018, 10102-10114, Islam *et al,* 2017, 319-332). In addition, location for keep and transportation process cause of modified temperature effected to water quality (Colt & Fornshell, 2014, 1-6).

Therefore, logistic regression model high TCB sample and impact of environmental variables on southern Thailand is challenging. TCB standards for drinking water are designed to guarantee limited disease risks (less than 2.2/100 milliliters). If more than standard it indicated a public health problem. The conclusion in this study environmental factors about year of production, air temperature and RMSC laboratory in Southern Thailand, we have found that high TCB sample with increased air temperature in drinking water in sealed containers and anticipate the pathogens similarly to indicator bacteria, which health risk will increase with air temperature and each laboratory. Wherewith all laboratories effected by difference factors the suitability of model for predict probability high TCB sample with calculation under the ROC curve less than 0.8, it is indicating poor harmony of the subject and not suitable for use (Lim. 2021). This may be due to an under-processing factor, too little study year of production (2015

– 2020), or incomplete consideration of environmental factors, this causes the resulting subject to have low harmony. So that, to optimize the model, the duration of the study about year of production should be increase and add another environmental factor relevant to make the model more efficient and suitable for predict water quality. However, there are many environmental factors, which were not included in this study. Many factors possibly effect TCB in water e.g., precipitation (Islam *et al,* 2017, 319-332, Seo *et al*, 2019, 1-16), season (Bojarczuk *et al*, 2018, 10102-10114). In the future all parameters must be included.

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VITAE

Name Miss Kritsanee Ruangsombat

Student ID 6310930061

Educational Attainment

Degree	Name of Institution	Year of Graduation
Bachelor (Biology)	Prince of Songkla University	2001
Master (Technology and Environmental Management)	Prince of Songkla University	2012

Scholarship Awards during Enrolment

Coastal Oceanography and Climate Change Research center (COCC), Faculty Environmental Management, Prince of Songkla University, Hat Yai, Songkhla, Thailand

Work – Position and Address

Position: Medical Scientist, Professional Level

Address: Regional Medical Sciences Center 11 Surat Thani, Department of Medical Sciences, Ministry of Public Health, Muang, Surat Thani, Thailand

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