

**MICROBIAL CONTAMINATION, RESISTANCE, AND HEAVY
METAL ANALYSIS OF BLOOD COCKLE (*Tegillarca granosa*, L.) IN
PANGUIL BAY, PHILIPPINES**

A RESEARCH PAPER

**Presented to
the Faculty of the College of Medical Technology
Misamis University
Ozamiz City**



**In Partial Fulfillment
of the Requirements for the Degree
BACHELOR OF SCIENCE IN MEDICAL TECHNOLOGY**

**JAMAICA L. ACAPULCO
ANGIE LEE G. LUNGAY
LANCE TRISTAN D. MAGHINAY
AARON CHARLES OPADA
GLYKA CRIZAN MAE B. PILAPIL**

June 2026



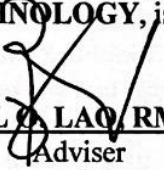
Misamis University
Ozamiz City
COLLEGE OF MEDICAL TECHNOLOGY



DEPARTMENT OF MEDICAL TECHNOLOGY

CERTIFICATE OF PANEL APPROVAL

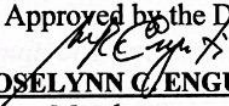
The thesis/research paper attached hereto, entitled “**MICROBIAL CONTAMINATION, RESISTANCE, AND HEAVY METAL ANALYSIS OF BLOOD COCKLE(*Tegillarca granosa*, L.) IN PANGUIL BAY, PHILIPPINES**”, prepared and submitted by **JAMAICA L. ACAPULCO, ANGIELEE G. LUNGAY, LANCE TRISTAN D. MAGHINAY, AARON CHARLES OPADA, and GLYKA CRIZAN MAE B. PILAPIL**, in partial fulfillment of the requirements for the degree **BACHELOR OF SCIENCE IN MEDICAL TECHNOLOGY**, is hereby recommended for approval.


KARL MAXEL C. LAO, RMT, MSc. MLS

Adviser

7/1/26
Date

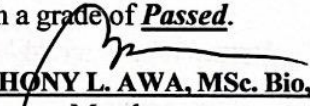
Approved by the Defense Panel Committee with a grade of ***Passed.***


MARIE ROSELYNN C. ENGUITO, MSc. Bio

Member

7/15/26

Date


ANTHONY L. AWA, MSc. Bio, LPT

Member

7/17/26

Date

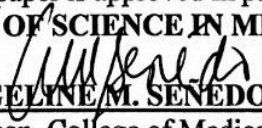

YUNALYN L. VILLANTES, Ph. D.

Chairman

7/17/26

Date

This thesis/research paper is approved in partial fulfillment of the requirements for the degree of **BACHELOR OF SCIENCE IN MEDICAL TECHNOLOGY**.


EVANGELINE M. SENEDO, RMT, MATMRS

Dean, College of Medical Technology

7/17/26

Date

ABSTRACT

Blood cockles (*Tegillarca granosa*, L.) are vital food and economic resources in Northern Mindanao and serve as bioindicators of environmental quality due to their filter-feeding nature. While Panguil Bay plays an important role in supporting livelihoods and food security, there has been limited up-to-date information assessing the combined impact of anthropogenic activities on microbial and heavy-metal contamination in local seafood resources, creating uncertainty regarding their safety for consumption and potential health risks. This study aimed to evaluate the extent of bacterial contamination, antibiotic resistance, and arsenic levels in blood cockles collected from Panguil Bay, Philippines. A descriptive-experimental research design was employed, in which samples underwent microbial isolation, biochemical identification, antibacterial susceptibility testing, and colorimetric analysis to determine arsenic. Results revealed the presence of *Escherichia coli* in all samples, with high microbial loads reaching up to 5.50×10^7 CFU/g in Tangub and 1.35×10^8 CFU/g in Baroy, while *Salmonella spp.* was not detected. Nitrate levels were low (0.1 mg/L) in both sampling sites, indicating that chemical parameters did not correspond with microbial contamination. Antibiotic susceptibility testing showed 100% resistance to ampicillin, whereas Ceftriaxone and Ciprofloxacin demonstrated the highest relative intermediate activity with mean zones of 17.50 mm and 15.84mm, respectively. Arsenic concentrations were below 0.05 mg/kg in all samples, remaining within acceptable safety limits. Overall, the findings indicated that although arsenic contamination was minimal, the high microbial load and observed antibiotic resistance posed potential health risks to consumers.

Keywords: *antibiotic resistance, arsenic, contamination, food-borne, health hazard*

DEDICATION

*This study is wholeheartedly dedicated to our beloved families,
whose unconditional love, understanding, sacrifices, and support have
been our constant source of strength and inspiration throughout the completion
of this research.*

*We also dedicated this work to our beloved adviser, Mr. Karl Maxel O Lao.
and to our research instructor, Dr. Nelfa D. Canini
for sharing their knowledge, guidance, and expertise, which
greatly contributed to the success of this study. Your patience
and encouragement inspired us to strive for excellence in the field of medicine
and scientific research.*

*and most especially, this research is dedicated to healthcare professionals,
researchers, environmental advocates, and concerned citizens who continuously
work toward protecting public health and food safety*

*May this study on the microbial contamination, antibacterial resistance,
and heavy metal analysis of Blood Cockle (*Tegillarca granosa*) in Panguil Bay
serves as a contribution to the promotion of environmental awareness, marine conservation, and
the safeguarding of human health*

*May we continue to value scientific research as a tool for protecting
both people and the environment for the present and future generations.*

Researchers

ACKNOWLEDGMENT

The researchers would like to express their heartfelt gratitude and sincerest appreciation to all the people and institutions who extended their support, guidance, and assistance in the completion of this study.

First and foremost, to our **Almighty God**, for granting us wisdom, strength, guidance, protection, and perseverance throughout the conduct of this research. His endless blessings gave us the courage to overcome every challenge encountered along the way.

To our beloved **families**, for their unconditional love, understanding, financial and moral support, encouragement, and prayers. Their unwavering faith in us became our inspiration and motivation in pursuing and completing this study successfully.

To **Mr. Karl Maxel O. Lao**, our research adviser, for his valuable guidance, patience, expertise, constructive suggestions, and encouragement that greatly contributed to the improvement and completion of this study.

To **Dr. Nelfa D. Canini**, our research instructor, for sharing her knowledge, guidance, support, and insightful recommendations that helped strengthen this research.

To the **Bureau of Fisheries and Aquatic Resources (BFAR)**, for their assistance in the identification of species used in this study and for their valuable contribution to the accuracy of the research.

To the **College of Medical Technology**, especially **Ma'am Ozzy and Sir Ankel**, for their generous assistance, patience, and support in the use of laboratory materials and equipment necessary for the conduct of this study.

To the **faculty and staff of the College of Arts and Sciences-Department of Natural Sciences Building**, whose laboratory facilities and accommodations were very critical in the successful completion of the experiments and analyses involved in this research.

Finally, to all individuals who, in one way or another, contributed their time, effort, knowledge, and support toward the realization of this study, the researchers extend their deepest gratitude.

Thank you very much!

Researchers

TABLE OF CONTENTS

	Page
TITLE PAGE	i
APPROVAL SHEET	ii
ABSTRACT	iii
DEDICATION	iv
ACKNOWLEDGMENT	v
TABLE OF CONTENTS	vii
LIST OF FIGURES	ix
LIST OF TABLES	x
LIST OF APPENDICES	xi
INTRODUCTION	
Background of the Study	1
Objectives of the Study	3
Significance of the Study	4
Scope and Delimitations	5
RESEARCH METHODOLOGY/MATERIALS AND METHODS	
Research Design	6
Research Environment	6
Sample Species Identification	7
Sample Collection, Storage, and Transport	8
Heavy Metal Analysis	9
Nitrate Determination	9
Homogenization and Serial Dilution for Bacterial Isolation	10
Presumptive, Confirmatory, and Confirmed tests for Colony Count	11
Isolation and Identification of Escherichia coli	12
Isolation and Identification of Salmonella spp	13
Antimicrobial Susceptibility Testing	14
Data Analysis	15
Statistical Analysis	16
Biosafety, Biosecurity, and Biowaste Management	17
Quality Control	17
RESULTS AND DISCUSSION	
Bacterial Analysis and Colony Counting	19
Antimicrobial Susceptibility Testing	22

Arsenic Contamination		23
Statistical analysis		25
CONCLUSION AND RECOMMENDATION		33
DECLARATION OF THE USE OF AI TOOLS		34
REREFERENCES CITED		35
APPENDICES		44
CURRICULUM VITAE		67

LIST OF FIGURES

Figure No.	Title	Page
Figure 1	Location map of sampling sites in Tangub City and Lanao del Norte	7
Figure 2	<i>Tegillarga granosa</i> (Linnaeus,1758)	8

LIST OF TABLES

Table No.	Title	Page
Table 1	Antibacterial Susceptibility Guide in Interpretation	15
Table 2	Microbial concentrations of <i>Escherichia coli</i> and <i>Salmonella</i> in two sampling sites	20
Table 3	Nitrate Analysis from two different sampling areas.	21
Table 4	Percent resistance in <i>E. coli</i> isolates in two different sampling areas.	22
Table 5	Arsenic results from two different sampling areas	24
Table 6.1	Descriptive statistics and Estimated Bacterial Density of Blood cockle samples	26
Table 6.2	Independent Samples t-Test Comparing Mean Bacterial Counts Between Tangub and Baroy samples	27
Table 6.3	Estimated Bacterial Density expressed as Colony-Forming Unity per Millimeter(CFU/mL)	28
Table 6.4	Antibiotic Susceptibility Test (Mean Zone of Inhibition,mm)	29
Table 7	Antibiotic Effectiveness Ranking (Overall Mean ZOI)	31

LIST OF APPENDICES

Appendix	Title	Page
APPENDIX A	Informed Consent	44
APPENDIX B	Approved Letter of Consent	45
APPENDIX C	Letter of Species Identification	52
APPENDIX D	Species Identification Results	53
APPENDIX E	Documentation	55
APPENDIX F	Raw Results	60
APPENDIX G	Turnitin and Grammarly results	

INTRODUCTION

Background of the Study

For millions of people worldwide, marine and coastal environments provide abundant food, livelihoods, and ecological services. 500 million people worldwide rely on small-scale fisheries, of whom 54 million fish mainly for their own food (FAO, 2024). Additionally, according to FAO (2020), Asia is the largest region for fishermen and aquaculture workers, accounting for 85% of the global total. Furthermore, over 250 million people in Southeast Asia alone depend on fish for at least 20% of their average daily animal protein consumption (Pomeroy et al., 2020). In the Philippines, almost 1.1 million municipal fishers fished for capture in 2023. In that year, there were more than two million fishermen in the nation (Philippines: Number of Municipal Fisherfolk by Livelihood, 2024).

Seafood has been considered one of the most essential parts of their diet. For every Filipino, a meal consists of rice and vegetables, and it is not complete without fish on the table (Ocean Philippines, 2022; Marine Stewardship Council, 2014). Aside from fish, one of the most popular is the clam. A wide variety of clams and other shellfish are abundant due to the country's long coastline and abundant marine life (Hou et al., 2023).

Blood cockles (*Tegillarca granosa*, L.) serve as an important food source and economic commodity in the country. Blood cockles (*T. granosa*, L.) are filter feeders that continuously strain large volumes of water to obtain food, making them excellent bioindicators for assessing environmental quality. However, this same biological process also makes them prone to accumulating pollutants, including microorganisms and toxic

heavy metals, which may threaten both marine biodiversity and human health (Almashhadany et al., 2024).

Heavy metals, commonly referred to as heavy metalloids, are metals with relatively large densities and atomic weights. These heavy metals mainly originate from industrial activities, mining, agricultural practices, and the combustion of fossil fuels. Because of their toxicity, persistence, bioaccumulation, and biomagnification, they pollute aquatic environments. Through bioaccumulation, which is a natural process in most bivalves, including blood cockles (*T. granosa*, L.), these heavy metals will be acquired (Almashhadany et al., 2024). Generally, exposure to these heavy metals can cause neurological, renal, and developmental problems in humans (Bosch et al., 2015). Arsenic is one of the most common metalloids found in the marine environment. Historically, it is known as “the king of poisons and poison of kings” for its lethal potency, which was used to kill many rivals of the ruling king. Arsenic has a significant impact on marine life and on human consumption. Seafood that contains arsenic is the main route of access to the human body. Once ingested by humans, it will affect the liver due to its strong toxicity, and it is also carcinogenic to the skin, bladder, and lungs (Kalia & Khambholja, 2023).

Shellfish, including blood cockles (*T. granosa*, L.), not only accumulate heavy metals but also microbes during bioaccumulation, resulting in microbial contamination, a long-recognized global safety concern. Foodborne diseases remain a major public health issue worldwide, with shellfish often harboring various bacteria such as *Escherichia coli* (*E. coli*), *Salmonella spp.*, and *Vibrio spp.* (Iwamoto et al., 2010).

In Northern Mindanao, Panguil Bay is among the most important fishing grounds, supporting livelihoods and food supplies for nearby cities, municipalities, and provinces across three administrative regions: Regions IX, X, and XII (Jimenez et al., 2009). However, the bay is increasingly exposed to various forms of pollution due to agricultural runoff, domestic sewage, aquaculture practices, and small-scale industrial activities (Sam et al., 2024). While Panguil Bay plays an important role in supporting livelihoods and food security in Northern Mindanao, there is a lack of up-to-date studies that assess the combined impact of anthropogenic activities on microbial and heavy-metal contamination in local seafood resources. In particular, to determine the contamination levels in blood cockles (*T. granosa* L.), despite their importance as a food source. It leaves uncertainties about how safe it is to consume and its potential health risks in the region.

Objectives of the Study

This study aimed to evaluate the extent of bacterial and heavy-metal contamination in blood cockles (*Tegillarca granosa*, L.) from Panguil Bay, Philippines. Specifically, this study :

1. Determined the presence of food-borne bacteria in blood cockles collected from Brgy. Maloro, Tangub City, and Brgy. Baroy Daku, Municipality of Baroy, Lanao Del Norte, through bacterial analysis with physicochemical analysis- nitrate analysis;

2. Quantified the number of bacterial colonies (*E. coli* and *Salmonella* spp.) that grow on various culture media by calculating the colony-forming units (CFUs);
3. Assessed the antibiotic susceptibility pattern of the isolates from the soft tissue of blood cockles using antimicrobial susceptibility testing (AST) and measured the zone of inhibition (ZOI) for various antibiotics; and
4. Determined the level of arsenic contamination in the soft tissue of the blood cockles using Silver Diethyldithiocarbamate Colorimetric Methods.

Significance of the Study

This study evaluates the microbiological quality and chemical safety of blood cockles (*T. granosa*, L.) harvested from Panguil Bay, offering critical data to safeguard public health and guide regional policy. By establishing baseline evidence on pathogen prevalence and antimicrobial resistance, the findings equip public health officials with the data necessary to accurately monitor foodborne hazards. Furthermore, the documented levels of bacterial contamination serve as a scientific foundation for Local Government Units (LGUs) and regulatory agencies to develop or enforce specific sanitation ordinances and environmental programs within the bay. Beyond policy applications, this research heightens local consumer awareness regarding the health risks of consuming raw or untreated bivalves, fostering safer food handling practices. Finally, this work expands the existing literature on marine environmental quality, serving as a reliable reference point for future researchers investigating food safety, pollution prevention, and the progression of antimicrobial resistance in aquatic ecosystems.

Scope and Delimitations

The study focused mainly on the microbial and heavy-metal analyses of blood cockles (*T. granosa*, L.) collected from Panguil Bay, Philippines. The scope of the study includes the identification and quantification of selected pathogenic microorganisms (*Escherichia coli* and *Salmonella spp*) and the heavy metal arsenic in the samples. Laboratory-based experimental methods were employed to generate quantitative data, which were then analyzed to determine the safety of the shellfish for human consumption.

The study was limited to blood cockle samples collected in selected areas of Panguil Bay during the specified collection period. Other shellfish species or aquatic organisms in the bay were not included in the analysis. The research is further limited to *Escherichia coli* and *Salmonella enterica*. While heavy metal contamination encompasses various elements, this study was strictly restricted to the assessment of arsenic; other heavy metals, alongside potential contaminants such as pesticides, fungi, microplastics, and organic pollutants, fell outside the scope of this investigation. Likewise, the research did not cover seasonal variations, long-term monitoring, or broader ecological impacts of contamination in Panguil Bay. These limitations are recognized as conditions beyond the researcher's control, such as environmental factors (tides, weather conditions, or water quality fluctuations) that may influence contamination levels. Despite these constraints, the study's scope is sufficient to provide meaningful data on microbial and heavy-metal contamination in blood cockles, thereby supporting its purpose of contributing to food safety, public health awareness, and sustainable fisheries management.

MATERIALS AND METHODS

Research Design

The study employed a descriptive-experimental research design to assess microbial and heavy-metal contamination in blood cockles (*T. granosa*, L.) collected from Panguil Bay, Philippines.

The descriptive part of the design focused on identifying the microbial load that provides a clear picture of the microbial contamination of blood cockles harvested from the bay. The experimental component involved laboratory antibacterial susceptibility testing of the isolated *E. coli* strains, to evaluate its antibacterial susceptibility pattern. The combined descriptive and experimental design was selected because it enables both qualitative and quantitative assessment, providing reliable data on the safety of blood cockles for human consumption and the potential health risks they may pose to the local population.

Research Environment

The research was conducted in Panguil Bay, specifically in Brgy. Maloro, Tangub City, Misamis Occidental (8.064453, 123.762457) and Brgy. Baroy Daku, Municipality of Baroy, Lanao del Norte (8.024728, 123.772800), Philippines, is an area where blood cockles (*T. granosa*, L.) are usually harvested (see Figure 1). The areas are characterized by small-scale shellfish harvesting activities, proximity to mangrove ecosystems, and influence from nearby riverine and irrigation discharges, which contribute to nutrient-rich sediments favorable for cockle growth.

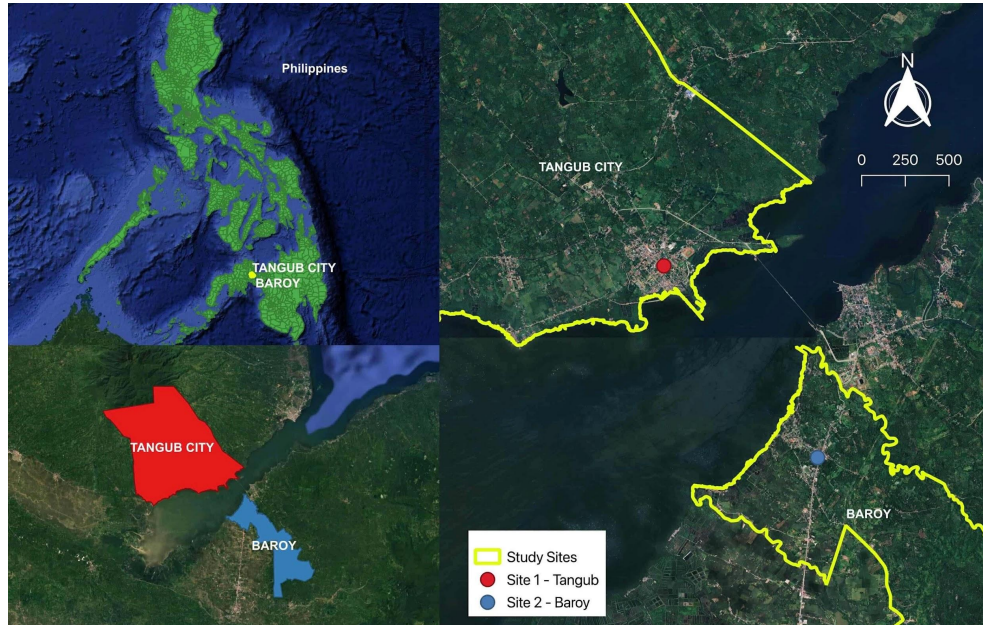


Figure 1. Sampling sites in Tangub City, Misamis Occidental, and the Municipality of Baroy, Lanao Del Norte

Sample Species Identification

Prior to sample collection and testing, species identification was conducted. Samples collected from Maloro, Tangub City, and the Municipality of Baroy, Lanao Del Norte, were sent to the Bureau of Fisheries and Aquatic Resources (BFAR)-Region X office in Cagayan de Oro City for morphological analysis of the shellfish (see Figure 2).



Figure 2. *Tegillarca granosa* (Linnaeus, 1758)

Sample Collection, Storage, and Transport

Three kilograms of blood cockles (*T. granosa*, L.) were collected from each site, for a total of 6 kilograms. From each location, 1 kg was used for heavy-metal analysis, and the remaining 2 kg for bacterial analysis.

After collection, the samples were placed in sterile plastic bags to prevent contamination during storage and transport. The samples were then placed in a cooler containing packed ice to maintain the temperature at 2 to 4 degrees Celsius (Shin & Moon, 2005; Hossen et al., 2014) and were transferred to the laboratory within 4 hours of collection. To ensure the samples are stored at the exact temperature, an infrared thermometer (Thermo gun) was used to monitor the temperature (Parikh & Rosenberg, 2013). The collected sample was rinsed with distilled water to remove mud, sand, and other visible debris before long-term storage and shell opening for soft-tissue extraction.

This is to avoid cross-contamination from the external part of the sample (Cheesbrough, 2006).

Heavy Metal Analysis

The analysis was conducted by the F.A.S.T laboratory in Cagayan de Oro City using Silver Diethyldithiocarbamate Colorimetric Methods outlined in AOAC International, 21st, 2019. A total of two (2) kg of blood cockles (*T. granosa*, L.), 1 kg from two separate sources, were tested. The blood cockles were opened using stainless steel forceps and a sterile knife to extract the soft tissue inside. One hundred (100) grams of blood cockle's soft tissue from two sites was sent to the F.A.S.T laboratory, totalling 200 grams. The sample was freeze-dried at -80 degrees Celsius for 24 hours to make it easier to grind into a homogeneous powder (Hossen et al., 2014). A clear solution was prepared to digest approximately 1 g of homogenized tissue using 2 mL of 30% hydrogen peroxide and 5 mL of concentrated nitric acid. After digestion, it was diluted with deionized water, and metal-specific color-developing reagents were added to create a colorful complex. The concentration of metals was measured using a spectrophotometer at 540 nm and compared with a standard calibration curve. Quality control samples and reagents with blanks were included to ensure accuracy (Hitachi High-Tech Corporation, 2024).

Nitrate Determination

Nitrate concentration was determined using the United States Environmental Protection Agency (US EPA) Method 352.1- Nitrogen, Nitrate (Colorimetric, Brucine

Method). This method is based on the reaction of nitrate ions with brucine sulfate in a concentrated sulfuric acid medium, producing a yellow-colored complex. The intensity of the color that is formed is directly proportional to the nitrate concentration present in the sample and is measured spectrophotometrically at a wavelength of 410nm (Method 352.1: Nitrogen, Nitrate (Colorimetric, Brucine) by Spectrophotometer, 1971) .

100mL of seawater samples were collected in sterile glass bottles from both sampling sites, then it is filtered to remove suspended particles before the analysis using a 0.45 μm membrane filter. Appropriate nitrate standards were prepared to generate a calibration curve. A 10-mL aliquot of the filtered sample was then mixed with 10 mL of sulfuric acid reagent prepared by carefully adding 500 mL of concentrated sulfuric acid to 125 mL of distilled water and allowing the mixture to cool.

Subsequently, 0.5 mL of brucine- sulfanilic acid reagent was added to each sample. The mixture was heated in a boiling water bath at 100 degrees celsius for 25 minutes, cooled to 10 to 15 degrees celcius, and the absorbance was measured at 410nm using a UV visible spectrophotometer against a reagent blank. Nitrate concentrations were determined from a calibration curve prepared using potassium nitrate standard make from 0.7218g anhydrous KNO_3 diluted to 1 L with distilled water and expressed as $\text{mg NO}_3^- \text{-N/L}$ (Holty & Potworowski, 1972; Jenkins & Medsker, 1964)

Homogenization and Serial Dilution for Bacterial Isolation

A total of 2 kilograms of fresh blood cockles (*T granosa*, L.), 1 kilogram from each location, was used for bacterial analysis. The samples were aseptically opened to extract the soft tissue using sterile stainless-steel forceps and a sterile knife (Hossen et al.,

2024). The extracted soft tissues were temporarily placed in a sterile 250 mL beaker (Veflen & Teixeira, 2022)

From the extracted soft tissues, 25 grams of blood cockle's soft tissues were aseptically weighed and blended with 225 mL of phosphate-buffered saline for 2 minutes using a blender with a sterile blade. After blending, 10 grams of the blended samples were homogenized with 9ml of 0.9% saline solution (Microbiology Society, 2024; Yulistiani et al., 2021).

After homogenization, serial dilutions were prepared separately to isolate *Salmonella* and *Escherichia coli* (E. coli). Six serial 10-fold dilutions were prepared by transferring 1 mL of the homogenate, using a sterile pipette, to a test tube containing 9 mL of 0.9% NaCl solution to make the first 1:10 dilution. Five additional dilution steps followed this; each was prepared by transferring 1 mL, using a sterile pipette, from the previous tubes into another tube containing 9 mL of sterile saline. Each tube was mixed thoroughly using a vortex mixer to ensure even distribution of microorganisms (Parikh & Rosenberg, 2013). To avoid contamination during the process, the test tubes were sealed with cotton plugs (Microbiology Society, 2024).

Presumptive, Confirmatory, and Completed tests for colony count

The presumptive, confirmatory, and completed test conducted by Yulistiani et al. (2021) was adapted. The presumptive test aimed to detect the possible presence of coliform bacteria by their ability to ferment lactose. Using sterile pipettes, 1 mL of the serially diluted homogenates was transferred and inoculated into three separate 5 mL test tubes containing lactose broth. After inoculation, the sterile Durham tubes were incubated

for up to 48 hours at 35 degrees Celsius. After incubation, they were checked for any gas formation within the Durham tube, indicating a positive result. If no gas formation occurs within 24 hours, the incubation is continued for up to 48 hours. This test detects whether the coliform is fermentative, meaning it ferments lactose, producing gas (Yulistiani et al., 2021).

The positive tube showing gas undergoes confirmatory testing. One loopful of culture was taken from each tube of positive lactose broth and placed into a tube of brilliant green lactose bile (BGLB) using a sterile loop, then the tube was incubated at 37 degrees Celsius for 48 hours. After incubation, the tubes were checked for any gas production; if so, the confirmatory test was positive.

Right after the confirmatory test, a completed test was performed on the bacterial colonies on Eosin Methylene Blue Agar (EMB Agar). The prepared medium was autoclaved at 121°C for 15 minutes to sterilize. After sterilization, it was allowed to cool before being poured aseptically onto sterile Petri dishes at about 20 mL per plate. Once the medium solidified, inoculation followed.

Isolation and Identification of *Escherichia coli*

A sterile loopful of bacterial culture from the confirmatory test was streaked onto another EMB plate in a quadrant streak pattern. Plates were inverted and incubated at 37 degrees Celsius for 24 to 48 hours. EMB agar inhibits gram-positive bacteria and supports the growth of Gram-negative enterics such as *Escherichia coli*. After incubation, the colony morphology was observed. Typical colonies appear as dark purple colonies with black centers. *E. coli*, on the other hand, shows a metallic green sheen on the surface

of the medium; this is because of the strong acid production, which precipitates the dyes (Yulistiani et al., 2021).

E. coli was further identified using the IMViC test. The test includes four tests: indole, methyl red, Voges-Proskauer, and citrate. *E. coli* will test positive for the indole and methyl red tests, showing a red ring in the indole test and a red color in the methyl red test, indicating that there is acid production. A negative result for the Voges-Proskauer and Citrate tests, with no color change in both, meaning it does not produce acetoin and cannot use citrate as its carbon source. These reactions confirmed the presence of *E. coli* among the bacterial isolates (Igbonezu et al., 2024).

Isolation and Identification of *Salmonella*

A sterile loopful of the bacterial suspension was aseptically streaked onto *Salmonella-Shigella Agar* (SSA) plates using the quadrant streaking method to obtain isolated colonies. The SSA, which contains bile salts and brilliant green dye, was selectively inhibited by Gram-positive and non-pathogenic Gram-negative organisms while differentiating lactose fermenters from non-fermenters based on colony color and hydrogen sulfide (H₂S) production (Gracias & McKillip, 2004).

For isolate identification, *Salmonella enterica* was identified using biochemical tests: Triple Sugar Iron (TSI), urease, and citrate tests (Farmer et al., 1985). *Salmonella enterica* was tested on Triple Sugar Iron, showing an alkaline (red) slant and an acid (yellow) butt, with H₂S production. A negative result for urease, with no change in the color of the medium, and a positive result for citrate. These reactions confirmed the

presence of *Salmonella spp.* among the bacterial isolates (Andrews et al., 2018; Forbes, 2007).

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility profile of *E. coli* and *Salmonella spp.* Isolates were determined using the Kirby–Bauer disc diffusion method on Mueller-Hinton Agar (MHA), following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2023; Humphries, 2018). A standard suspension of confirmed isolates of *Salmonella* species and *E. coli* was inoculated onto Mueller-Hinton (MH) agar plates. The isolates were tested against commercially available antibiotic discs, which include ampicillin, gentamicin, ceftriaxone, ciprofloxacin, and chloramphenicol. These antibiotics were selected based on their availability and in accordance with World Health Organization recommendations (CLSI, 2020).

A standard bacterial inoculum corresponding to 0.5 McFarland turbidity was prepared for each isolated bacterium using the method described by Hudzick (2009). Using sterile isolation, isolated colonies were collected and suspended in 3 mL of saline. It was adjusted to 0.5 McFarland turbidity by visual comparison.

A dry, sterile swab was dipped into the inoculum tube. It was rotated against the tube's side. It was then streaked on the entire surface of the MHA plate. After plating, the antibiotic disks were placed on the agar surface within 15 minutes of inoculation using sterile forceps. The disks were placed 24mm (center-to-center) apart to avoid overlapping zones. Once all disks are in place, cover the agar late, and were incubated invertedly at 35 degrees celcius for 16 to 18 hours (Padmaja, 2025).

After incubation, the zone of inhibition was measured using a vernier caliper to the nearest millimeter. The measured zones of inhibition for each isolate were interpreted and categorized as Susceptible (S), Intermediate (I), or Resistant (R). These classifications were strictly based on the Clinical and Laboratory Standards Institute standard reference thresholds for Enterobacteriaceae as detailed in the table below.

Table 1. Antimicrobial Susceptibility Guide in Interpretation

Antibiotic	Susceptible (S)	Intermediate (I)	Resistant (R)
Ampicillin	$\geq 17\text{mm}$	14-16mm	$\leq 13\text{mm}$
Ciprofloxacin	$\geq 21\text{mm}$	16-20mm	$\leq 15\text{mm}$
Ceftriaxone	$\geq 23\text{mm}$	20-22mm	$\leq 19\text{mm}$
Chloramphenicol	$\geq 18\text{mm}$	13-17mm	$\leq 12\text{mm}$
Gentamicin	$\geq 15\text{mm}$	13-14mm	$\leq 12\text{mm}$

Note: Interpretation of susceptibility (S), intermediate (I), and resistance (R) was based on the standardized breakpoint ranges adapted from CLSI (2011).

Data Analysis

Quantitative and descriptive information was acquired through microbial isolation, colony counting, biochemical testing, and antimicrobial susceptibility profiling, which was used to examine the contamination of *Escherichia coli* and *Salmonella spp.* in blood cockle (*Tegillarca granosa*, L.) sampled from Maloro, Tangub City, and the Municipality of Baroy, Lanao Del Norte.

To quantify microbial load, colony counts from culture plates were computed to obtain the colony-forming units per gram (CFU/g) after getting the average using the formula (Dumaloan-Canini et al., 2024) :

$$\text{CFU/g} = \frac{\text{Number of Colonies Counted} \times \text{dilution factor}}{\text{Volume plated}} \quad \text{Equation (1)}$$

For antibacterial susceptibility testing, the diameters of the zone of inhibition (in mm) were measured for each plate. The percentage of resistance was calculated using the formula (Dumaloan-Canini et al., 2024) :

$$\text{Resistance (\%)} = \left(\frac{\text{Number of Resistant Isolates}}{\text{Total number of Isolates tested}} \right) \times 100 \quad \text{Equation (2)}$$

Statistical Analysis

Data normality was verified using the Shapiro-Wilk test to ensure the bacterial counts followed a normal distribution.

For the antimicrobial susceptibility test (AST), results were analyzed both descriptively and statistically. The mean and standard deviation of the zones of inhibition (ZOI) were calculated for each antibiotic, and isolates were classified as Susceptible (S), Intermediate (I), or Resistant (R) based on the Clinical and Laboratory Standards Institute (CLSI, 2011) guidelines. To determine if a significant difference existed between the microbial densities and ZOI values of the two sampling sites, an Independent Samples T-test was employed. All statistical analyses were performed at a 0.05 level of significance.

Biosafety, Biosecurity, and Biowaste Management

All procedures involving biological samples were done under Biosafety Level 2 (BSL-2) conditions with appropriate personal protective equipment (PPE). Work areas and tools were disinfected with 70% ethanol (Gracias & McKillip, 2004). Cultures were disinfected with Lysol and autoclaved at 121°C for 15-20 minutes after testing to ensure microbial inactivation (Tang et al., 2025). Antimicrobial susceptibility testing was performed in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. Antibiotic discs were handled with sterile forceps, and media were stored at proper temperatures. Biowaste such as agar plates, pipette tips, swabs, used and unused antibiotic disc, and gloves was collected in labeled biohazard bags, autoclaved, and disposed of in accordance with Misamis University-College of Medical Technology biosafety protocols. Liquid waste was chemically treated before disposal. All procedures adhered to international biosafety standards (World Health Organization, 2022; Nagy et al., 2018).

Quality Control

To ensure the validity and reproducibility of microbiological results, stringent quality control measures were implemented throughout the study. A standard reference strain, *Escherichia coli* ATCC 25922, obtained from the American Type Culture Collection (ATCC), was used as the positive control organism. This strain is internationally recognized as a quality control organism for antimicrobial susceptibility testing and general microbiological procedures due to its stable phenotypic profile and predictable response patterns (Minogue et al., 2014).

The positive control was processed under identical experimental conditions as the test samples to verify the performance of culture media, reagents, and incubation conditions. The consistent and expected growth characteristics of *E. coli* ATCC 25922 served as an indicator that laboratory procedures were properly executed. The use of standardized control strains is essential in minimizing variability and ensuring comparability of antimicrobial testing results across laboratories, particularly in susceptibility assays and microbiological evaluations (Jepson et al., 2016).

RESULTS AND DISCUSSION

Bacterial Analysis and Colony Counting

The analysis of blood cockles (*Tegillarca granosa*) collected in Tangub City and Baroy confirmed the presence of *E. coli* in all samples, as confirmed by growth on Eosin Methylene Blue (EMB) agar. Ritu Pasrija & Kumari (2025) confirmed that methods based on plating or on using specific agar types for plate counts, such as *E. coli* on EMB agar, help confirm the presence of *E. coli* in food. The second dilution was selected for final analysis as it yielded countable colonies within the statistically valid range of 30–300 colony-forming units. Moreover, serial dilutions are a valuable tool for colony counting and an essential component for accurately analyzing microbial populations (Ferguson, 2024).

The study demonstrated that samples from Tangub City posed the greatest potential risk (see Table 2), with peak microbial loads significantly higher than those from Baroy. Conversely, all samples tested negative for *Salmonella* spp., indicating that while general fecal contamination was high, this specific pathogen was not detected during the sampling period. The presence of these pathogens calls for consistent monitoring and improved sanitary controls to ensure the safety of blood cockles for public consumption. According to Tansinmarshall (2025), the number of microbes present in various locations can indicate either the sources of that contamination or the environmental conditions that enable the microbes to multiply. The author also pointed out that the usual method for calculating microbiological density is the arithmetic mean because it allows a direct comparison between locations.

The average concentration of *Escherichia coli* at both sampling locations significantly exceeded the safety criteria of ≤ 230 MPN/100g established by EU Regulation (EC) No 2073/2005, a contemporary gold standard for products placed on the market (Bua et al., 2022). While these levels also surpass the historical allowable limit of 2.3×10^2 CFU/mL proposed by Hood et al. (1983), the modern EU framework provides a more stringent benchmark for assessing the severe microbial contamination observed. Specifically, concentrations reached 2.08×10^6 CFU/mL in Tangub and a markedly higher 2.77×10^6 CFU/mL in Baroy.

Table 2. Microbial concentrations of *Escherichia coli* and *Salmonella* in two sampling sites

Area	Bacterium	Average Concentration (CFU/mL)	Permissible Limit (EU Regulation)
Tangub	<i>Escherichia coli</i>	2.08×10^6 CFU/mL	2.3×10^2 CFU/mL
Baroy	<i>Escherichia coli</i>	2.77×10^6 CFU/mL	2.3×10^2 CFU/mL
Tangub	<i>Salmonella spp.</i>	0×10^6 CFU/mL	2.3×10^2 CFU/mL
Baroy	<i>Salmonella spp.</i>	0×10^6 CFU/mL	2.3×10^2 CFU/mL

Note: CFU/g, Colony Forming Unit/milliLiter; *Escherichia coli* concentrations are expressed in CFU/mL

Chemical analysis of seawater at both sampling sites (see Table 3) yielded a uniform nitrate concentration of 0.1 mg/L (NO₃-N). When evaluated against the standing Water Quality Guidelines in DAO No. 2016-08, these values are significantly lower than the 10 mg/L permissible limit for Class SA waters (DENR, 2016). However, the corresponding microbial analysis showed alarming *E.coli* concentrations of 2.08×10^6 CFU/g in Tangub and 2.77×10^6 CFU/g in Baroy. This indicates that chemical compliance does not equal biological safety.

Table 3. Nitrate analysis from two different sampling areas

Area	Nitrate Concentration(mg/L)	Permissible Limit (mg/L) (DENR, 2016)
Tangub	0.1mg/L	10 mg/L
Baroy	0.1mg/L	10 mg/L

Note: mg/L, milligrams/Liter

When oxygen is depleted or limited in water matrices, *E.coli* dynamically shifts its metabolism from aerobic respiration to anaerobic respiration (Cole & Richardson, 2008). In the absence of oxygen, *E.coli* preferentially utilizes environmental nitrate as a terminal electron acceptor to support its metabolic functions and promote cell growth (Jones et al., 2011; Potter, 2000). During this respiratory process, *E.coli* executes a catalytic, dissimilatory reduction that directly converts available nitrate into nitrite (Tiso & Schechter, 2015). This enzymatic conversion provides a distinct growth advantage, allowing the bacterial population to multiply rapidly while actively consuming and depleting the baseline nitrate pool in the water system.

The low nitrate levels suggest a lack of significant agricultural runoff, yet the high microbial load confirms recent fecal contamination. This shows that chemical compliance does not equal biological safety. Nitrate is a poor indicator of recent fecal contamination (Pearl et al., 2024). Bivalves bioaccumulate pathogens even in chemically clean water (Rochete-Newall et al., 2023). This highlights the dangerous limitation of relying solely on chemical parameters to monitor shellfish harvesting areas, as cockles effectively bioaccumulate pathogens even in chemically passing waters.

Antimicrobial Susceptibility Testing

The antibacterial susceptibility patterns of *E. coli* isolates from different sampling locations, as shown in Table 4, were determined using the Kirby-Bauer disk diffusion method. The zones of inhibition were interpreted based on standardized breakpoints to classify isolates as susceptible, intermediate, or resistant.

The results show the highest level of resistance to ampicillin, with 100% of *E. coli* isolates consistently having low or zero zones of inhibition (0-10mm), were categorized as resistant (see Table 3). This suggests that ampicillin is mainly useless against the isolates in this study. Beta-lactamase enzymes are frequently linked to resistance because they hydrolyze beta-lactam antibiotics, making them ineffective (Kapoor et al., 2017)

Table 4. Percent resistance in *E. coli* isolates in two different sampling areas.

Antibiotics	Percent Resistance (%)
Ampicillin	100%
Ciprofloxacin	58.3%
Ceftriaxone	41.7%
Chloramphenicol	33.3%
Gentamicin	75%

Note: Percent resistance (%) was derived by dividing the number of *Escherichia coli* isolates classified as resistant to a specific antibiotic by the total number of isolates tested, then multiplying by 100.

For ciprofloxacin, the result showed varying susceptibility: some isolates showed wide zones of inhibition, indicating susceptibility, while others showed smaller zones, indicating resistance. Additionally, a few of the isolates were in the intermediate range.

This implies that although resistance is already developing in the population, ciprofloxacin is still effective against some bacterial strains (CLSI, 2011).

Ceftriaxone also showed varying reactions in isolates. Many isolates showed a narrower zone of inhibition, indicating resistance, although some showed wide zones of inhibition consistent with susceptibility. This variability indicates that ceftriaxone is not always effective and that resistance may be emerging among the isolates

Results with chloramphenicol were inconsistent; some isolates had moderate to high zones of inhibition, while others had little zones of inhibition. This suggests different degrees of susceptibility, which could be caused by resistance mechanisms such as efflux pumps or enzymatic inactivation (Livermore et al., 2011)

The response to gentamicin was variable, with some showing moderate susceptibility and others demonstrating resistance. The presence of aminoglycoside-modifying enzymes or decreased membrane permeability, which can restrict antibiotic uptake, may be the case of heterogeneity (Becker & Cooperr, 2012; Magnet & Blanchard, 2005)

Environmental factors, such as bacterial adaptation and genetic mutation in *Escherichia coli* isolates from shellfish, may contribute to the development of resistance to antibiotics (Sahoo et al., 2010)

Arsenic Contamination

As shown in Table 5 arsenic concentration throughout all samples from the two locations remained below 0.50 mg/kg, a value much lower than the maximum permitted limit of 0.05 mg/kg for inorganic arsenic in bivalve mollusks defined by Commission

Regulation (European Union), 2025. Given that the blood cockle samples easily meet the most recent and strict safety limits established by the European Union, this suggests that there is very little toxicological risk associated with heavy metal contamination.

Table 5. Arsenic results from two different sampling areas

Area	Arsenic Concentration(mg/L)	Permissible Limit (European Union, 2025))
Tangub	< 0.05 mg/kg	0.50 mg/kg
Baroy	< 0.05 mg/kg	0.50 mg/kg

Note: Arsenic (As) content was expressed in mg/kg and determined from shellfish samples collected in two sampling areas.

In ecotoxicological monitoring of coastal environments, multi-element tracking in bivalve molluscs typically shows that essential metals required for metabolic functions bioaccumulate at the highest absolute tissue concentrations, establishing a common hierarchy where Iron (Fe) and Zinc (Zn) consistently rank as the top two most abundant elements, followed by Manganese (Mn) and Copper (Cu) (Anthony Anani & Ovie Olomukoro, 2020; Jolaosho et al., 2024). While these essential elements are physiological requirements for shellfish, non-essential metalloids like Arsenic (As) require dedicated scientific investigation due to their extreme toxicity at trace levels (Jolaosho et al., 2024). Arsenic was specifically selected for this research because it represents a high-risk, chronic food safety threat that conventional abundance rankings fail to capture. While essential nutrients possess metabolic pathways for regulation, non-essential metalloids like arsenic possess persistent, long-term bioaccumulation potential within the soft tissues of filter-feeding bivalves (Ochoa-Esteso et al., 2024; Xu & Yu, 2024).

Several environmental and biological factors may influence the absence of elevated levels in all samples. One of the major factors is the area's quality, as arsenic contamination in shellfish is strongly linked to environmental exposure. Coastal waters that are less affected by industrial contamination, mining activities, and agricultural runoff tend to have lower arsenic concentrations (Smedley & Kinniburgh, 2012). Thus, the low arsenic levels observed in this study may suggest that the sampling area is relatively unpolluted by this heavy metal.

Additionally, arsenic in seafood is commonly present in organic forms, such as arsenobetaine, which are significantly less toxic than inorganic arsenic (Sakurai, 2002). This explains why even when arsenic is detected in marine organisms, it does not necessarily cause a serious health risk at low concentrations. The ability of bivalves, such as the chosen sample, *Tegillarga granosa*, L., to accumulate arsenic depends on the environmental factors that highly influence the arsenic mobility and uptake (Maher et al., 2018)

Statistical Analysis

Bacterial Load In Blood Cockle Samples Across Sampling Sites

The bacterial counts observed in the blood cockle samples varied between the two sampling sites (see Table 6.1), indicating differences in the level of microbial contamination present in the collected shellfish. Among the sites, Baroy recorded the highest mean bacterial load of 257.40 CFU with a Log10 mean of 6.41, while Tangub showed a slightly lower mean count of 207.80 CFU and a Log10 mean of 6.32. Both

sampling sites were interpreted as having a “Very High” bacterial load, suggesting considerable microbial contamination in the shellfish samples.

Table 6.1. Descriptive Statistics and Estimated Bacterial Density of Blood Cockle Samples

Sample	Mean Colony Count (CFU)	Standard Deviation (SD)	Variance	Log 10 Mean (CFU/mL)
Tangub	207.80	28.41	807.00	6.32
Baroy	267.40	41.69	1733.00	6.41

Note: CFU, Colony Forming Unit; Reference: 230 E. coli / 100 g of shellfish flesh

The higher standard deviation and variance observed in Baroy further indicate greater variability in bacterial concentration, which may be influenced by environmental factors such as water quality, organic matter accumulation, and anthropogenic activities. These findings imply that the shellfish harvested from both areas were exposed to contamination sources, supporting the findings of Kaufman et al. (2003), who reported that shellfish can accumulate high bacterial loads due to their filter-feeding behavior.

In the Philippines, Peralta and Andalecio (2011) reported that bacterial counts in shellfish-growing waters can range from 50 to 9,200 bacteria per 100 milliliters, indicating that high microbial loads are common in coastal aquaculture environments and may pose potential health risks.

The bacterial counts of the sampling locations in Tangub City and Baroy were compared using an independent sample t-test to see if there was a statistically significant difference (see Table 6.2). There was a significant difference in the mean bacterial

densities between the two locations. The results indicate that the location of the sampling site has a significant influence on the microbial load present in the blood cockle.

As a result, the null hypothesis, which posited that there is no significant difference in bacterial abundance between the two sampling sites, is rejected. This suggests that the bioaccumulation of *E. coli* in both sampling sites is influenced differently by localized environmental factors or different levels of fecal contamination.

Table 6.2. Independent Samples t-Test Comparing Mean Bacterial Counts Between Tangub and Baroy

Source of Variation	df	t-value	p-value	Decision
ETB2 vs ETD2	8	2.24	0.055	Fail to reject Ho

Note: Significant @ $p > 0.05$

The results show that both of the samples contain relatively high levels of bacterial contamination, raising concerns about their overall safety and quality. According to established microbial guidelines, bacterial counts above 100 CFU/mL are considered elevated, while those exceeding 1000 CFU/mL are classified as very high risk (Alshishani et al., 2026). In this case, both of the samples (ETB2, ETD2) fall within this very high range, suggesting a substantial presence of microorganisms.

Estimated bacterial density (CFU/mL) was calculated from the mean colony counts obtained through plate count enumeration, where the average number of colonies was converted into colony-forming units per milliliter (CFU/mL) based on the dilution factor used during analysis (see Table 6.3). The interpretation of “High Risk” was based on comparison with standard microbiological quality guidelines, where elevated CFU/mL

values indicate significant bacterial contamination and potential health risk, as commonly applied in environmental microbiology assessments following standard plate count methods.

Table 6.3. Estimated Bacterial Density Expressed as Colony-Forming Units per Milliliter (CFU/mL)

Sample	Mean Colony Count	Estimated CFU/mL
Tangub	207.80	2.08×10^6 CFU/mL
Baroy	257.40	2.57×10^6 CFU/mL

Note: CFU/mL, Colony Forming Unit/ Milliliter.

These findings may indicate issues such as poor handling practices, contamination during processing, or ineffective treatment methods. While the analysis only measures total bacterial load and does not identify specific pathogens, higher counts are often associated with an increased likelihood of harmful microorganisms being present. According to the World Health Organization, elevated microbial levels in water are linked to increased public health risks, particularly when they exceed recommended safety limits (WHO, 2017). Overall, the results highlight the need for improved sanitation practices, stricter handling procedures, and regular monitoring to help reduce contamination and ensure safety.

The antibiotic susceptibility results (see Table 6.4) demonstrated varying responses of the bacterial isolates to the antibiotics tested. Overall, ceftriazone and ciprofloxacin were the most effective antibiotics, producing the largest zones of inhibition and indicating strong antibacterial activity. In contrast, ampicillin showed the

least effectiveness, with little to no inhibition observed in several samples, particularly samples collected from Baroy. This pattern suggest that there is a difference in susceptibility among the bacterial isolates and indicates the presence of antibiotic resistant strains within the sampling environments.

Table 6.4. Antibiotic Susceptibility Test (Mean Zone of Inhibition, mm)

Sample	AMP	CIP	C	CRO	CN
ETB2	7.00 ± 6.56	17.00 ± 7.21	14.33 ± 11.50	17.67 ± 5.03	7.33 ± 5.77
ETD2	9.33 ± 1.15	14.67 ± 0.58	15.00 ± 1.73	17.33 ± 6.51	8.33 ± 3.51

Note: AMP, Ampicillin; CIP, Ciprofloxacin; C, Chloramphenicol; CRO, Ceftriaxone; CN, Gentamicin

Among the antibiotics tested, ceftriaxone consistently exhibited the highest antibacterial activity, achieving the greatest mean zone of inhibition in the samples collected in Baroy (27.67 ± 3.21 mm). This indicates that the majority of the bacteria in the samples remain susceptible to these medications. Significant effectiveness was shown by ciprofloxacin, supporting its well-established status as a broad-spectrum antibiotic. Antibiotics like ciprofloxacin and ceftriaxone remain effective against environmental bacteria because they inhibit critical bacterial functions, such as DNA replication and cell wall synthesis, thereby hindering bacterial survival (Cabello, 2006).

Gentamicin and chloramphenicol have moderate and variable effects, inhibiting some bacterial isolates while showing reduced activity against others. This is consistent with the notion that bacterial populations are diverse rather than one type. While some strains have already developed tolerance to the medications, others are still susceptible. This trend was described by Zemb (2010), who observed that bacteria can exchange

resistance genes through plasmids in the environment, allowing resistance to spread among many species.

The poor performance of ampicillin, particularly the complete resistance observed in some samples, further supports the presence of resistant bacterial strains in the aquatic environment. The differences in susceptibility patterns among locations suggest that bacterial populations are not uniform and may be influenced by local environmental conditions, pollution levels, and other ecological stressors.

The most effective antibiotic was ceftriaxone, which is promising but should be interpreted cautiously. Although it did well in this experiment, research from around the world has already demonstrated that bacteria are progressively developing resistance to more potent antibiotics. According to Husna et al. (2014), extended-spectrum β -lactamase (ESBL)-producing bacteria are becoming more prevalent in the environment, which means that if resistance persists, even medications like ceftriaxone may eventually lose their effectiveness.

The differences between samples also support the idea that bacterial populations are not consistent. Since these samples came from the same locations, the changes in the results suggest that some bacteria are dominant at different locations. For example, the complete resistance to ampicillin in Tangub shows that resistant strains can become more noticeable under certain conditions. This agrees with findings from environmental studies indicating that aquatic bacterial populations are heterogeneous and influenced by pollution levels and environmental stressors.

The antibiotic effectiveness ranking (see Table 7) summarizes the overall susceptibility patterns of the bacteria isolated based on mean zone sizes. Ceftriaxone has

the highest mean ZOI(17.50mm), making it the most effective antibiotic, followed by Ciprofloxacin (15.84mm), Chloramphenicol (14.67mm), Gentamicin (7.83mm), and Ampicillin (8.17mm), which was the least effective.

Table 7. Antibiotic Effectiveness Ranking (Overall Mean ZOI)

Antibiotics	Mean ZOI (mm)	S (CLSI, 2011)	I (CLSI, 2011)	R (CLSI, 2011)	Effectiveness
Ceftriaxone	17.50mm	≥ 23mm	20-22mm	≤ 19mm	Most Effective
Ciprofloxacin	15.84mm	≥ 21mm	16-20mm	≤ 15mm	Highly Effective
Chloramphenicol	14.67mm	≥ 18mm	13-17mm	≤ 12mm	Moderate
Gentamicin	8.17mm	≥ 17mm	14-16mm	≤ 13mm	Low
Ampicillin	7.83mm	≥ 15mm	13-14mm	≤ 12mm	Least Effective

Note: Interpretation of susceptibility (S), intermediate (I), and resistance (R) was based on the standardized breakpoint ranges adapted from CLSI (2011).

Mean Zone of Inhibition (ZOI) was measured in millimeters (mm) using the Kirby-Bauer disk diffusion method to assess the antibacterial effectiveness of different antibiotics against *Escherichia coli* isolates. Antibiotics with larger inhibition zones were classified as more effective, indicating stronger bacterial growth inhibition, whereas smaller zones indicated reduced effectiveness or resistance of the isolates.

Ceftriaxone and Ciprofloxacin are the most effective antibiotics, whereas Ampicillin showed possible resistance. The ranking clearly shows that the bacterial isolates are highly susceptible to Ceftriaxone and Ciprofloxacin, moderately susceptible to Chloramphenicol, poorly susceptible to Gentamicin, and strongly resistant to Ampicillin.

In contrast to similar studies, the pattern aligns with Cabello (2006), who found that β -lactam antibiotics such as Ampicillin were the least effective against aquatic bacterial isolates, with mean inhibition zones often below 10 mm due to the presence of β -lactamase and other resistance mechanisms. Miranda and Zemelman (2002) reported a similar investigation and found that, in general, environmental isolates exhibited larger inhibition zones (around 15–25 mm) with Ciprofloxacin, confirming its high efficiency in this study. For Chloramphenicol and Gentamicin, the moderate-to-low effectiveness observed in this study is also consistent with Zemb (2010), who reported intermediate inhibition zones in environmental bacterial populations, often due to heterogeneous resistance genes carried by different bacterial strains. This explains the variability in susceptibility and the reduced effectiveness compared to Ciprofloxacin and Ceftriaxone.

CONCLUSION AND RECOMMENDATION

The study confirmed the presence of *Escherichia coli* in *Tegillarca granosa* L. collected from Panguil Bay, indicating fecal contamination in the sampling sites. Although *Salmonella* spp. was not detected, the recorded microbial loads exceeded the acceptable safety limits, suggesting poor microbiological quality were found to be within permissible levels. The bacterial isolates exhibited resistance to ampicillin but remained susceptible to ceftriaxone and ciprofloxacin. Overall, the findings highlight microbiological safety concerns associated with shellfish harvested from the study area.

Future researchers are encouraged to expand the scope of the study by including other pathogenic microorganisms, particularly *Vibrio* spp, to obtain a more comprehensive evaluation of microbial contamination in shellfish. Additional analyses involving other heavy metals, environmental pollutants, and seasonal variations are also recommended to assess further shellfish safety and the environmental quality of Panguil Bay. The applications of molecular and advanced analytical techniques are suggested to improve sanitation and waste management practices, and enforce stricter regulations on shellfish harvesting and handling. Public awareness initiatives regarding proper seafood preparation and safe shellfish consumption should also be promoted to minimize potential health risks among consumers.

DECLARATION OF THE USE OF AI TOOLS

We acknowledge the use of **ChatGPT, Quilbot, and Grammarly** in assisting with the preparation of this research paper. The AI tool was used to help improve sentence construction, grammar, clarity, and conciseness of the manuscript.

Prompt: We entered the following prompt/s...

“ Revise the sentences to focus on the antibiotic resistance in *E.coli* isolates from shellfish.”

“ Improve the clarity and grammar of this paragraph.”

“Paraphrase this sentence using formal academic language.”

“Help me understand the table.”

Use: We used and modified the output to improve the grammar, revise sentence structure, and enhance the clarity and organization of our paper.

REFERENCES CITED

- Adl-Doust, S., Becerril, M. N., Feng, P., & Hui, T. (2025). Bacterial spread in public washrooms: A comparative analysis of hand dryers, paper towels, and ambient air. *Expedition*, 2(1).
- Andrews, W. H., Jacobson, A., Ge, B., Zhang, G., & Hammack, T. (2018, May). *FDA.gov archive*. U.S. Food and Drug Administration. <https://www.fda.gov/about-fda/about-website/fdagov-archive>
- Anthony Anani, O., & Ovie Olomukoro, J. (2020). Assessment of Metal Accumulation and Bioaccumulation Factor of Some Trace and Heavy Metals in Freshwater Prawn and Crab. *Crustacea*. <https://doi.org/10.5772/intechopen.88103>
- Armstrong, R. A., & Hilton, A. C. (2010). Statistical analysis in microbiology: StatNotes. Wiley-Blackwell.
- Alshishani, A., Al-ebini, Y., Shoai, M., Alsafadi, D., Hussein, G., & Shaghlil, L. (2026). Quality and safety assessment of bottled water in Jordan markets. *Journal of Food Composition and Analysis*, 151, 108929. <https://doi.org/10.1016/j.jfca.2026.108929>
- Almashhadany, D. A., Rashid, R. F., Altaif, K. I., Mohammed, S. H., Mohammed, H. I., & Al-Bader, S. M. (2024). Heavy metal(loid) bioaccumulation in fish and its implications for human health. *Italian Journal of Food Safety*. <https://doi.org/10.4081/ijfs.2024.12782>
- Becker, B., & Cooper, M. A. (2012). Aminoglycoside Antibiotics in the 21st Century. *ACS Chemical Biology*, 8(1), 105–115. <https://doi.org/10.1021/cb3005116>
- Bosch, A. C., O'Neill, B., Sigge, G. O., Kerwath, S. E., and Hoffman, L. C. (2015). Heavy metals in marine fish meat and consumer health: a review. *Journal of the Science of Food and Agriculture*, 96(1), 32–48. <https://doi.org/10.1002/jsfa.7360>
- Bua, A., Piras, F., Cossu, M., Mazza, R., Delogu, A. M., & Consolati, S. G. (2022). Association between *Escherichia coli* and *Salmonella* spp. food safety criteria in live bivalve molluscs from wholesale and retail markets. *Food Control*, 137, 108929. <https://doi.org/10.1016/j.foodcont.2022.108942>
- Cabello, F. C. (2006). Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environmental Microbiology*, 8(7), 1137–1144. <https://doi.org/10.1111/j.1462-2920.2006.01054.x>

- Cole, J. A., & Richardson, D. J. (2008). Respiration of Nitrate and Nitrite. *EcoSal Plus*, 3(2). <https://doi.org/10.1128/ecosal.3.2.5>
- Cheesbrough, M. (2006). *District Laboratory Practice in Tropical Countries*. <https://doi.org/10.1017/cbo9780511543470>
- CLSI (2020). <https://www.nih.org.pk/wp-content/uploads/2021/02/CLSI-2020.pdf>
- CLSI (2011). Zone diameters of antimicrobial agents according to CLSI guidelines 2011. Zone diameters are in mm. Antimicrobial agent Disc content When testing Susceptible, Intermediately susceptible Resistant. https://www.microrao.com/micronotes/pg/kirby_bauer.pdf
- Clinical and Laboratory Standards Institute. (2023). Performance standards for antimicrobial disk susceptibility tests; Approved standard (13th ed., CLSI document M02) and Performance standards for antimicrobial susceptibility testing (33rd ed., CLSI supplement M100). Wayne, PA: CLSI.
- Commission Regulation (EU) 2025/1891 of 17 September 2025 amending Regulation (EU) 2023/915 as regards maximum levels of inorganic arsenic in fish and other seafood. Official Journal of the European Union, L series. <http://data.europa.eu/eli/reg/2025/1891/oj>
- Department of Environment and Natural Resources. (2016). Water quality guidelines and general effluent standards of 2016 (Administrative Order No. 2016-08). <https://pab.emb.gov.ph/wp-content/uploads/2017/07/DAO-2016-08-WQG-and-GES.pdf>
- Dumaloan-Canini, N., Cadelinia, E. E., & Olarte-Bala, J. J. (2024). Occurrence of Potentially Pathogenic Bacteria in Commercially Sold Seafood from Misamis Occidental, Philippines. *European Journal of Nutrition & Food Safety*, 16(2), 42–51. <https://doi.org/10.9734/ejnf/2024/v16i21386>
- FAO. (2020). *The State of World Fisheries and Aquaculture 2020*. [Www.fao.org](http://www.fao.org). <https://www.fao.org/interactive/state-of-fisheries-aquaculture/2020/en/>
- FAO. (2024). *FAO Knowledge Repository*. [Fao.org. https://openknowledge.fao.org/items/22b0e37f-1785-4404-8743-5a44bc276674](https://openknowledge.fao.org/items/22b0e37f-1785-4404-8743-5a44bc276674)
- Farmer, J. J., Davis, B. R., Hickman-Brenner, F. W., McWhorter, A., Huntley-Carter, G. P., Asbury, M. A., Riddle, C., Wathen-Grady, H. G., Elias, C., & Fanning, G. R. (1985). Biochemical identification of new species and biogroups of Enterobacteriaceae isolated from clinical specimens. *Journal of Clinical Microbiology*, 21(1), 46–76. <https://doi.org/10.1128/jcm.21.1.46-76.1985>

- Ferguson, M. (2024, October 22). *APC: Aerobic Plate Count for Serial Dilutions*. R-Project.org.
https://cran.r-project.org/web/packages/MPN/vignettes/b_apc-vignette.html
- Forbes, B. A. (2007). *Study guide for bailey & scott's Diagnostic Microbiology, Twelfth edition*. Elsevier Mosby.
- Gracias, K. S., & McKillip, J. L. (2004). A review of conventional detection and enumeration methods for pathogenic bacteria in food. *Canadian Journal of Microbiology*, 50(11), 883–890. <https://doi.org/10.1139/w04-080>
- Hitachi High-Tech Corporation. (2024). 4. *Colorimetric Analysis* (3). <https://www.hitachi-hightech.com/global/en/knowledge/analytical-systems/spectrophotometers/uv/basics/course4.html#:~:text=Generally%2C%20in%20absorptiometry%20where%20a,to%20determine%20the%20sample%20concentration.>
- Holty, J. G., & Potworowski, H. S. (1972). Brucine method for the determination of nitrate in water. *Environmental Science & Technology*, 6(9), 835-837. https://www.epa.gov/sites/default/files/2015-08/documents/method_352-1_1971.pdf
- Hossen, Md. F., Hamdan, S., & Rahman, Md. R. (2014). Cadmium and lead in Blood Cockle (*Anadara Granosa*) from Asajaya, Sarawak, Malaysia. *The Scientific World Journal*, 2014, 1–4. <https://doi.org/10.1155/2014/924360>
- Hudzicki, J. (2009, December 8). Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. <https://asm.org/getattachment/2594ce26-bd44-47f6-8287-0657aa9185ad/kirby-bauer-disk-diffusion-susceptibility-test-protocol-pdf.pdf>
- Humphries, R. M. (2018). The continued value of disk diffusion for assessing antimicrobial susceptibility. *Journal of Clinical Microbiology*, 56(7), e00437–18. <https://doi.org/10.1128/JCM.00437-18>
- Husna, A., Rahman, M. M., Badruzzaman, A. T. M., Sikder, M. H., Islam, M. R., Rahman, M. T., Alam, J., & Ashour, H. M. (2023). Extended-Spectrum β -Lactamases (ESBL): Challenges and Opportunities. *Biomedicines*, 11(11), 2937. <https://doi.org/10.3390/biomedicines11112937>
- Igbonezu, V. N., Onoriode Christian Eruteya, Uguomore, E. O., Kingsley Chimuanya Umezurike, & Okonkwo, P. O. (2024). Proximate Analysis and Occurrence of Antibiotic Resistant *Escherichia coli* in Street Vended Ready to Eat Edible Larvae of *Rhynchophorus phoenicis* in Port Harcourt, Nigeria. *International Journal of Pathogen Research*, 13(4), 105–118. <https://doi.org/10.9734/ijpr/2024/v13i4303>

- Iwamoto, M., Ayers, T., Mahon, B. E., & Swerdlow, D. L. (2010). Epidemiology of seafood-associated infections in the United States. *Clinical Microbiology Reviews*, 23(2), 399–411. <https://doi.org/10.1128/cmr.00059-09>
- Jenkins, D., & Medsker, L. L. (1964). Brucine method for determination of nitrate in ocean, estuarine, and fresh waters. *Analytical Chemistry*, 36(3), 610–612. <https://pubs.acs.org/doi/10.1021/ac60209a016>
- Jepson, A. K., Schwarz-Linek, J., Ryan, L., Ryadnov, M. G., & Poon, W. C. K. (2016). What Is the “Minimum Inhibitory Concentration” (MIC) of Pexiganan Acting on *Escherichia coli*?—A Cautionary Case Study. *Advances in Experimental Medicine and Biology*, 33–48. https://doi.org/10.1007/978-3-319-32189-9_4
- Jimenez, J., De Guzman, A., Jimenez, C., & Acuña, R. (2009). Panguil Bay Fisheries over the Decades: Status and Management Challenges. *Journal of Environment & Aquatic Resources*, 1. <https://doi.org/10.48031/msunjea.2009.01.02>
- Jolaosho, T. L., Elegbede, I. O., Akintola, S. L., Jimoh, A. A., Ndimele, P. E., Mustapha, A. A., & Adukonu, J. D. (2024). Bioaccumulation dynamics, noncarcinogenic and carcinogenic risks of heavy metals in commercially valuable shellfish and finfish species from the world largest floating slum, Makoko, Nigeria. *Marine Pollution Bulletin*, 207, 116807. <https://doi.org/10.1016/j.marpolbul.2024.116807>
- Jones, S. A., Gibson, T., Maltby, R. C., Chowdhury, F. Z., Stewart, V., Cohen, P. S., & Conway, T. (2011). Anaerobic Respiration of *Escherichia coli* in the Mouse Intestine. *Infection and Immunity*, 79(10), 4218–4226. <https://doi.org/10.1128/iai.05395-11>
- Kalia, K., and Devang Bharatkumar Khambholja. (2023). Arsenic in the marine environment—Contents, speciation, and its biotransformation. *Elsevier EBooks*, 761–789. <https://doi.org/10.1016/b978-0-323-89847-8.00012-2>
- Kapoor, G., Saigal, S., & Elongavan, A. (2017). Action and resistance mechanisms of antibiotics: A guide for clinicians. *Journal of Anaesthesiology Clinical Pharmacology*, 33(3), 300–305. https://doi.org/10.4103/joacp.joacp_349_15
- Kaufman, G. E., Bej, A. K., DePaola, A., & others. (2003). Oyster-to-oyster variability in levels of *Vibrio parahaemolyticus*. *Applied and Environmental Microbiology* <https://doi.org/10.4315/0362-028X-66.1.125>
- Keyence Cooperation. (2025). *Manual colony counting vs Automated Colony counters*. <https://www.keyence.com/products/microscope/colony-counter/resources/colony-counter-resources/manual-colony-counting-vs-automated-colony-counters.jsp#:~:text=Counting%20colonies%20manually%20has%20the,strain%2C%20and%20variability%20between%20users>.

- Kuivenhoven, M., & Mason, K. (2020). *Arsenic toxicity*. PubMed; StatPearls Publishing. <https://www.ncbi.nlm.nih.gov/books/NBK541125>
- Livermore, D. M., Warner, M., Mushtaq, S., Doumith, M., Zhang, J., & Woodford, N. (2011). What remains against carbapenem-resistant Enterobacteriaceae? Evaluation of chloramphenicol, ciprofloxacin, colistin, fosfomycin, minocycline, nitrofurantoin, temocillin and tigecycline. *International Journal of Antimicrobial Agents*, 37(5), 415–419. <https://doi.org/10.1016/j.ijantimicag.2011.01.012>
- Magnet, S., & Blanchard, J. S. (2005). Molecular Insights into Aminoglycoside Action and Resistance. *Chemical Reviews*, 105(2), 477–498. <https://doi.org/10.1021/cr0301088>
- Maher, W., Waring, J., Krikowa, F., Duncan, E., & Foster, S. (2018). Ecological factors affecting the accumulation and speciation of arsenic in twelve Australian coastal bivalve molluscs. *Environmental Chemistry*, 15(2), 46. <https://doi.org/10.1071/en17106>
- Marine Stewardship Council. (2014, June 27). *Sustaining the fishing culture of the Philippines*. MSC International - English. <https://www.msc.org/media-centre/news-opinion/news/2020/02/25/sustaining-the-fishing-culture-of-the-philippines>
- Martinez, B. A., Bianchini, A., Stratton, J., Raabe, O., & Swanson, S. (2021). Condensation removal practices and their potential for contributing to environmental pathogen contamination in food processing facilities. *Journal of Food Protection*, 84(6), 1047–1054. <https://doi.org/10.4315/JFP-20-45>
- Martini, K. M., Boddu, S. S., Nemenman, I., & Vega, N. M. (2024). Maximum likelihood estimators for colony-forming units. *Microbiology Spectrum*, 12(9). <https://doi.org/10.1128/spectrum.03946-23>
- McMenemy, K., McLeod, C., & others. (2018). A predictive model for pathogen variability in shellfish. *Strathprints Repository*. <https://doi.org/10.1371/journal.pone.0193865>
- Method 352.1: Nitrogen, Nitrate (Colorimetric, Brucine) by Spectrophotometer. (1971). https://www.epa.gov/sites/default/files/2015-08/documents/method_352-1_1971.pdf
- Microbiology society. (2024) Basic practical microbiology A Manual <https://microbiologysociety.org/static/uploaded/23cbf9c5-f8c8-4f91-b092a4ad819e6357.pdf>
- Miranda, C. D., & Zemelman, R. (2001). Antibiotic Resistant Bacteria in Fish from the Concepción Bay, Chile. *Marine Pollution Bulletin*, 42(11), 1096–1102. [https://doi.org/10.1016/s0025-326x\(01\)00093-5](https://doi.org/10.1016/s0025-326x(01)00093-5)

- Minogue, T. D., Daligault, H. A., Davenport, K. W., Bishop-Lilly, K. A., Broomall, S. M., Bruce, D. C., Chain, P. S., Chertkov, O., Coyne, S. R., Freitas, T., Frey, K. G., Gibbons, H. S., Jaissle, J., Redden, C. L., Rosenzweig, C. N., Xu, Y., & Johnson, S. L. (2014). Complete Genome Assembly of *Escherichia coli* ATCC 25922, a Serotype O6 Reference Strain. *Genome Announcements*, 2(5), e00969-14. <https://doi.org/10.1128/genomeA.00969-14>
- Nagy, E., Boyanova, L., & Justesen, U. S. (2018). How to isolate, identify and determine antimicrobial susceptibility of anaerobic bacteria in routine laboratories. *Clinical Microbiology and Infection*, 24(11), 1139–1148. <https://doi.org/10.1016/j.cmi.2018.02.008>
- Oceana Philippines.(2022). *A fishy situation: Diving into the Pinoy's current fish and seafood consumption and the decline in marine resources*. (2022). <https://ph.oceana.org/press-releases/fins-study/>
- Ochoa-Esteso, C., Roselló-Carrió, A., Carrasco-Correa, E. J., & Lerma-García, M. J. (2024). Bioaccumulation of environmental pollutants and marine toxins in bivalve molluscs: a review. *Exploration of Foods and Foodomics*, 2, 788-809. <https://doi.org/10.37349/eff.2024.00062>
- Omorodion, N., & Emmanuel, I. (2022). Microbial diversity and nutritional profile of processed and unprocessed shellfish species. *ResearchGate Publication*. <https://doi.org/10.30574/wjbphs.2022.10.1.006>
- Padmaja, N., Purushottam, M. D., & Srilalitha, V. (2025). Prevalence of multi-drug resistant, extensively drug resistant, Pan drug resistant bacteria from intensive care units of a tertiary care teaching hospital, Amalapuram, India. *Bioinformation*, 21(06), 1475–1480. <https://doi.org/10.6026/973206300211475>
- Parikh, B. R., & Rosenberg, H. (2013). Temperature monitoring. *Anesthesia Equipment*, 295–306. <https://doi.org/10.1016/b978-0-323-11237-6.00014-5>
- Paerl, H. W., Dyble, J., Moisander, P. H., Noble, R. T., Piehler, M. F., Pinckney, J. L., Steppe, T. F., Twomey, L., & Valdes, L. M. (2003). Microbial indicators of aquatic ecosystem change: current applications to eutrophication studies. *FEMS Microbiology Ecology*, 46(3), 233–246. [https://doi.org/10.1016/s0168-6496\(03\)00200-9](https://doi.org/10.1016/s0168-6496(03)00200-9)
- Passariello, C., Di Nardo, D., Miccoli, G., De Biase, A., Gambarini, G., & Testarelli, L. (2019). Microbial contamination of brand new nickel-titanium endodontic instruments. *La Clinica Terapeutica*, 170(4), e258–e261. <https://doi.org/10.7417/CT.2019.2144>

- Peralta, E. M., & Andalecio, M. N. (2011). Microbiological quality of oyster (*Crassostrea* sp.) and mussel (*Perna viridis*) in selected growing areas in Western Visayas, Philippines. *Asian Fisheries Science*, 24, 237–247.
- Philippines: number of municipal fisherfolk by livelihood 2021. (2024, November). Statista.
<https://www.statista.com/statistics/1350775/philippines-number-of-municipal-fisherfolk-by-type-of-livelihood/>
- Pomeroy, R., Parks, J., and Green, G. (2020, January 27). *Fisheries Partnerships*. Indo-Pacific Defense Forum.
<https://ipdefenseforum.com/2020/01/fisheries-partnerships/>
- Potter, L. (2000). Novel growth characteristics and high rates of nitrate reduction of an *Escherichia coli* strain, LCB2048, that expresses only a periplasmic nitrate reductase. *FEMS Microbiology Letters*, 185(1), 51–57.
[https://doi.org/10.1016/s0378-1097\(00\)00070-7](https://doi.org/10.1016/s0378-1097(00)00070-7)
- Ritu Pasrija, & Kumari, D. (2025). *Counting Colony Forming Units (CFU) to Estimate Viable Fungal Cells in a Sample*. 53–61.
https://doi.org/10.1007/978-981-96-7047-5_3
- Rochelle-Newall, E., Nguyen, T. M. H., Le, T. P. Q., Sengtaheuanghoung, O., & Ribolzi, O. (2015). A short review of fecal indicator bacteria in tropical aquatic ecosystems: knowledge gaps and future directions. *Frontiers in Microbiology*, 6.
<https://doi.org/10.3389/fmicb.2015.00308>
- Sahoo, K. C., Tamhankar, A. J., Johansson, E., & Lundborg, C. S. (2010). Antibiotic use, resistance development and environmental factors: a qualitative study among healthcare professionals in Orissa, India. *BMC Public Health*, 10(1).
<https://doi.org/10.1186/1471-2458-10-629>
- Sakurai, T. (2002). Review: Biological effects of organic arsenic compounds in seafood. *Applied Organometallic Chemistry*, 16(8), 401–405.
<https://doi.org/10.1002/aoc.325>
- Sam, L., An C., Perico, C. M., Magdayao, E. D., and Acot, F. T. (2024). Macroplastic pollution in mangrove forests of Tanguib City, Panguil Bay, Philippines. *Nusantara Bioscience*, 16(2). <https://doi.org/10.13057/nusbiosci/n160212>
- Sharp, J. H., Clements, K., Diggins, M., McDonald, J. E., Malham, S. K., & Jones, D. L. (2021). *E. coli* Is a Poor End-Product Criterion for Assessing the General Microbial Risk Posed From Consuming Norovirus Contaminated Shellfish. *Frontiers in Microbiology*, 12. <https://doi.org/10.3389/fmicb.2021.608888>

- Shin, Y. K., & Moon, T.-S. (2005). Temperature tolerance and physiological changes of blood cockle, *Tegillarca Granosa*. *Korean Journal of Fisheries and Aquatic Sciences*, 38(4), 251–256. <https://doi.org/10.5657/kfas.2005.38.4.251>
- Smedley, P. L., & Kinniburgh, D. G. (2012). Arsenic in Groundwater and the Environment. *Essentials of Medical Geology*, 279–310. https://doi.org/10.1007/978-94-007-4375-5_12
- Tang, Q., Yan, F., Yuan, L., Tang, Y., Chen, H., Sun, Y., Yang, M., & Song, G. (2025, October 20). *Enhancing laboratory biosafety management: A comprehensive strategy from theory to practice*. *Frontiers*. <https://www.frontiersin.org/journals/public-health/articles/10.3389/fpubh.2024.1439051/full>
- Tamsinmarsh all4. (2025, April 3). *Means, Ranges and Replicates: Improving Microbial Plate Counting*. *EJPPS*. <https://www.ejpps.online/post/means-ranges-and-replicates-improving-microbial-plate-counting>
- Tiso, M., & Schechter, A. N. (2015). Nitrate Reduction to Nitrite, Nitric Oxide and Ammonia by Gut Bacteria under Physiological Conditions. *PLOS ONE*, 10(3), e0119712. <https://doi.org/10.1371/journal.pone.0119712>
- Twomey, C. L., Beitz, H., & Johnson, H. B. (2009). Bacterial contamination of surgical scrubs and laundering mechanisms: Infection control implications. *Infection Control Today*.
- Venegas Arques, M. C., Rojas García, C. P., Cataldo Saavedra, Y. A., Jiménez Gomez, P. F., Arqués Vergara, V. I., & Martinez, B. (2021). Bacterial contamination of dental aerosol with and without use of an acrylic dome in a patient in COVID-19 pandemic. *International Journal of Odontostomatology*, 15(1), 14–22. <https://doi.org/10.4067/S0718-381X202100010001>
- Veflen, N., & Teixeira, P. (2022). Food safety myths consequences for Health: A Study of reported gastroenteritis incidence and prevalence in UK, Norway and Germany. *Food Control*, 142, 109210. <https://doi.org/10.1016/j.foodcont.2022.109210>
- Whitehead, K., Pilkington, L. I., Slate, A. J., Saubade, F., Amin, M., Lutey, A., Gemini, L., Kling, R., & Romoli, L. (2023). The cleanability of laser etched surfaces with repeated fouling using *Staphylococcus aureus* and milk. *Food and Bioproducts Processing*, 137, 145–154. <https://doi.org/10.1016/j.fbp.2022.11.007>
- World Health Organization. (2022). *World health statistics 2022*. World Health Organization. <https://www.who.int/news/item/20-05-2022-world-health-statistics-2022>

- World Health Organization. (2017, April 24). *Guidelines for drinking-water quality, 4th edition, incorporating the 1st addendum*. Wwww.who.int. <https://www.who.int/publications/i/item/9789241549950>
- Yulistiani, R., Adi Saputro, E., & Raharjo, D. (2021). Bacterial contamination and cadmium heavy metal content of Blood Cockle (*Anadara Granosa*Linn) satay sold by street vendors in Surabaya, Indonesia. *E3S Web of Conferences*, 328, 01013. <https://doi.org/10.1051/e3sconf/202132801013>
- Zemb, O., West, N., Bourrain, M., Godon, J. J., & Lebaron, P. (2010). Effect of a transient perturbation on marine bacterial communities with contrasting history. *Journal of Applied Microbiology*, 109(3), 751–762. <https://doi.org/10.1111/j.1365-2672.2010.04706.x>

APPENDICES

APPENDIX A: Informed Consent

 **Misamis University**
Ozamiz City

MISAMIS UNIVERSITY RESEARCH CENTER
CERTIFIED: ISO 21001: 2018 Educational Organizations Management System by DNV
ACCREDITED: Philippine Association of Colleges and Universities Commission on Accreditation (PACUCOA)

MU-RC-042/13 November 2024

Thesis/Research Data Gathering Approval Form

Date: January 21, 2024

Name of Authors: Lance Tristan Maghirang, Gilda Pilapil, Angeline Lunday, Janice Arapula, Aaron Opada
College/Department: College of Medical Technology
Research Title: Microbial and Heavy Metal Analysis of Blood Cattle (Tegillarca granosa L.) in Pangasinan, Philippines

Data Gathering Procedure:

a. Type of Research: (Kindly check)

☐ Quantitative	☐ Qualitative	☒ Experimental
☒ Approved Research Title & Problem/Objectives	☒ Approved Data Gathering Procedure	☐ Approved Data Gathering Instrument/Data Sheets

b. Respondents/Participants/Subject/Samples to be Collected: Sample to be collected: Blood Cattle

c. Procedure:

☐ Survey	☐ Interview	☒ Experimentation	☒ Laboratory Testing
---------------------------------	------------------------------------	---	--

d. Place of Implementation/Study Area: Misamis University, Science Building

If to be conducted outside the school campus, indicate the nature of transportation to be used:

☐ Own private vehicle	☒ public utility vehicle	☐ rental	☐ Others, please indicate
--	--	---------------------------------	--

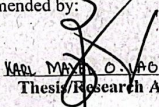
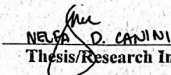
e. Date/s of Implementation/Sampling: February 13, 2024 onwards

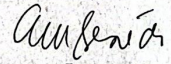
Attachments:

☐ Approved Letter of Consent from the designated authority in the area/place where the study is to be conducted
☐ Signed Parents' Consent

The thesis proposal mentioned above is hereby recommended for the commencement of data gathering implementation.

Recommended by:

 <u>KARL MAY JR. O. A.B., RMT, MSc, MEd</u> Thesis/Research Adviser	 <u>NELVA D. CANINA</u> Thesis/Research Instructor
--	---

Approved by: 
EVANGELINA M. SERVEDO, RMT, PHARM.S
College Dean

APPENDICES

APPENDIX B: Approved Letters of Consent

**Misamis University**
Ozamiz City
COLLEGE OF MEDICAL TECHNOLOGY
CERTIFIED: ISO 21001: 2018 Educational Organizations Management System by DNV
ACCREDITED: Philippines Association of Colleges and Universities Commission on Accreditation (PACUCOA)

January 22, 2026

DR. HAYDEE D. VILLANUEVA
Dean, College of Arts and Sciences
Misamis University

To Whom It May Concern,

Good day!

We are student researchers from the College of Medical Technology of Misamis University. We would like to respectfully ask permission to use the laboratory facilities and necessary laboratory equipment for our research entitled **"Microbial and Heavy Metal Analysis of Blood Cockle (*Tegillarca granosa*, L.) in Panguil Bay, Philippines."**

The laboratory will be used for the preparation and analysis of samples, including microbiological testing and heavy metal analysis, which are essential for the completion of our study. Access to the laboratory facilities will help us ensure proper procedures, accurate results, and reliable findings.

We assure you that all laboratory rules, safety measures, and standard protocols will be strictly followed at all times. We also commit to using all laboratory equipment carefully and responsibly and returning them in good condition after use.

Thank you very much for your time and kind consideration. We are hoping for your favorable approval.

Respectfully yours,


JAMAICA L. ACAPULCO
Researcher


AARON CHARLES L. OPADA
Researcher


ANGIE LEE C. LUNGAY
Researcher


LANCE TRISTAN D. MAGHINAY
Researcher

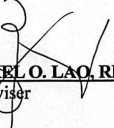

GLYKA CRIZAN MAE B. PILAPIL
Researcher

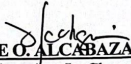
APPENDICES

APPENDIX B Cont'd

 **Misamis University** 
Ozamiz City
COLLEGE OF MEDICAL TECHNOLOGY

CERTIFIED: ISO 21001: 2018 Educational Organizations Management System by DNV
ACCREDITED: Philippines Association of Colleges and Universities Commission on Accreditation (PACUCOA)

Noted by: 
KARL MAXEL O. LAO, RMT, MSc. MLS
Research Adviser


ARJADE O. ALCABAZA, RChT
Laboratory In-Charge

Recommending Approval:

DR. NELFA D. CANINI
Chairperson, Department of Natural Sciences

Approved by:

DR. HAYDEE D. VILLANUEVA
Dean, College Of Arts and Sciences

APPENDICES

APPENDIX B Cont'd

 MISAMIS UNIVERSITY Ozamiz City Department of Student Affairs & Services
MU-DSAS-006/02June2023

PARENT'S CONSENT

Sponsoring Group/Organization

I am allowing my son/daughter/ward, JAMAICA L. ACAPULCO, to join the RESEARCH WRITING AND PRESENTATION (activity) to be held on JANUARY TO MAY 2024 at MISAMIS UNIVERSITY under the supervision of MR. KARL MAXEL O. LAO (Adviser/Faculty).

I understand that Misamis University exercises the necessary safety precautions in this activity.

In consideration of the benefits to be derived from the above activity, I expressly waive any and all claims against the administration or any member of the faculty and staff of the Misamis University on account of any unforeseen accident or injury that my son/daughter/ward might incur in connection with the aforementioned activity.

<u>EDNA L. ACAPULCO</u> Signature over printed name of Parent/Guardian	<u>JANUARY 20, 2024</u> Date
<u>JAMAICA L. ACAPULCO</u> Signature over printed name of Student	<u>JANUARY 20, 2024</u> Date

Important: This form shall be submitted to the DSAS Office at least two (2) days before the conduct of the activity.

JAN 21 2024

SUBSCRIBED AND SWORN to before me, this ____ day of ____ at OZAMIZ CITY, Philippines, that the herein affiant personally came and appeared with his/her _____, as evidence of his/her personal identity.

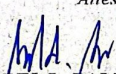
Doc. No. 424
Page No. 64
Book No. 1x
Series of 20 20

ATTY. REY E. SINAG, RMT
Notarial Commission No. 2025-18
Notary Public for Ozamiz City and Clarin
City Hall Drive, Bernad Subdivision, Aguada, Ozamiz City
PTR No. 6007751; 01/05/2026; Ozamiz City
IBP O.R. No. 578165; 12/29/2025; Pasig City
Attorney's Roll No. 70648
MCLE Compliance No. VIII-0014127; Valid until 12/31/2028

Notary Public

(For DSAS Personnel only)

Attested by:


RODEL L. BALDADO, MHist, MAEd
OIC, Department of Student Affairs & Services

Date: _____

APPENDICES

APPENDIX B Cont'd

	MISAMIS UNIVERSITY Ozamiz City Department of Student Affairs & Services
MU-DSAS-006/02June2023	

PARENT'S CONSENT

Sponsoring Group/Organization _____

I am allowing my son/daughter/ward, ANGIE LEE G. LUNGAY, to join the RESEARCH PAPER WRITING AND PRESENTATION (activity) to be held on JANUARY - MAY 2026 at MISAMIS UNIVERSITY under the supervision of KARL MAXEL D. LAD, RMT, MSc. MLs (Adviser/Faculty).

I understand that Misamis University exercises the necessary safety precautions in this activity.

In consideration of the benefits to be derived from the above activity, I expressly waive any and all claims against the administration or any member of the faculty and staff of the Misamis University on account of any unforeseen accident or injury that my son/daughter/ward might incur in connection with the aforementioned activity.

<u>MARIA EDEN G. LUNGAY</u> Signature over printed name of Parent/Guardian	<u>01/19/26</u> Date
<u>ANGIE LEE G. LUNGAY</u> Signature over printed name of Student	<u>01/19/26</u> Date

Important: This form shall be submitted to the DSAS Office at least two (2) days before the conduct of the activity.

SUBSCRIBED AND SWORN to before me, this 20 JAN 2026 day of JANUARY at OZAMIZ CITY, Philippines, that the herein affiant personally came and appeared with his/her _____, as evidence of his/her personal identity.

Doc. No. 245
Page No. 48
Book No. 48
Series of 20 26

ATTY. JASON CHINO MARAMBA
NOTARY PUBLIC
WASHINGTON ST., 5TH DISTRICT, OZAMIZ CITY
NOTARIAL COMMISSION NO. 1010 UNTIL FEBRUARY 28, 2027
PTR NO. 101092 DATE 07/07/2022
IBP NO. 101092 DATE 07/07/2022
ATTORNEY'S REG. NO. 82224 DATE 05/30/2022
MCLE COMPLIANCE NO. VIII-0044696 VALID UNTIL 04/14/2026
TIN No. _____
Roll No. _____

(For DSAS Personnel only)

Attested by:

RODEL C. BALDADO, MHist, MAEd
OIC, Department of Student Affairs & Services
Date: _____

APPENDICES

APPENDIX B Cont'd

	MISAMIS UNIVERSITY Ozamiz City Department of Student Affairs & Services
MU-DSAS-006/02 June 2023	

PARENT'S CONSENT

Sponsoring Group/Organization

I am allowing my son/daughter/ward, Lance Tristan D. Maghinay, to join the Research Writing and Presentation (activity) to be held on January to May at Misamis University under the supervision of Mr. Karl Maxel O. Lao (Adviser/Faculty).

I understand that Misamis University exercises the necessary safety precautions in this activity.

In consideration of the benefits to be derived from the above activity, I expressly waive any and all claims against the administration or any member of the faculty and staff of the Misamis University on account of any unforeseen accident or injury that my son/daughter/ward might incur in connection with the aforementioned activity.

 <u>MARGLOS D. MAGHINAY</u> Signature over printed name of Parent/Guardian	<u>JAN 26, 2026</u> Date
 <u>LANCE TRISTAN D. MAGHINAY</u> Signature over printed name of Student	<u>JAN 20, 2026</u> Date

Important: This form shall be submitted to the DSAS Office at least two (2) days before the conduct of the activity.

SUBSCRIBED AND SWORN to before me, this JAN 21 2026 day of JANUARY at OZAMIZ CITY, Philippines, that the herein affiant personally came and appeared with his/her _____, as evidence of his/her personal identity.

Doc. No. <u>423</u> Page No. <u>81</u> Book No. <u>1X</u> Series of 20 <u>24</u>	 ATTY. REY B. BINAG, RMT Notarial Commission No. 2025-18 Notary Public for Ozamiz City and Clarin City Hall Drive, Bernad Subdivision, Aguada, Ozamiz City PTR No. 6007751; 01/05/2025; Ozamiz City PTR No. _____ IBP O.R. No. 576165; 12/29/2025; Pasig City YBP No. _____ Attorney's Roll No. 70648 TIN No. _____ MCLE Compliance No. VPI-JU14127; Valid until 04/14/2028 Roll No. _____
---	--

(For DSAS Personnel only)

Attested by:


RODEL M. BALDADO, MHist, MAEd
OIC Department of Student Affairs & Services

APPENDICES

APPENDIX B Cont'd

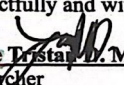
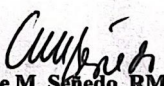
	MISAMIS UNIVERSITY Ozamiz City Department of Student Affairs & Services
MU-DSAS-006/02June2023	
PARENT'S CONSENT	
Sponsoring Group/Organization	
I am allowing my son/daughter/ward, <u>Aaron Charles L. Opada</u>, to join the <u>Research Writing and Presentation</u> (activity) to be held on <u>January to May 2026</u> at <u>Misamis University</u> under the supervision of <u>Hr. Karl Maxel O. Lao</u> (Adviser/Faculty). I understand that Misamis University exercises the necessary safety precautions in this activity. In consideration of the benefits to be derived from the above activity, I expressly waive any and all claims against the administration or any member of the faculty and staff of the Misamis University on account of any unforeseen accident or injury that my son/daughter/ward might incur in connection with the aforementioned activity.	
<u>Kathleen Ch. Alejandro</u> Signature over printed name of Parent/Guardian	<u>January 19, 2026</u> Date
<u>Aaron Charles L. Opada</u> Signature over printed name of Student	<u>January 19, 2026</u> Date
<i>Important: This form shall be submitted to the DSAS Office at least two (2) days before the conduct of the activity.</i>	
SUBSCRIBED AND SWORN to before me, this <u>JAN 21 2026</u> at <u>OZAMIZ CITY</u>, Philippines, that the herein affiant personally came and appeared with his/her _____, as evidence of his/her personal identity.	
Doc. No. <u>428</u> Page No. <u>86</u> Book No. <u>IX</u> Series of 20 <u>21</u>	ATTY. REY D. BINAG, RMT Notarial Commission No. 2025-18 Notary Public for Ozamiz City and Clarin City Hall Drive, Bernad Subdivision, Aguada, Ozamiz City PTR No. 6007751; 01/05/2026; Ozamiz City IBP O.R. No. 576165; 12/29/2025; Pasig City Attorney's Roll No. 70648 MCLC Compliance No. VIII-0014127; Valid until 04/14/2028 Notary Public _____ PTR No. _____ IBP No. _____ TIN No. _____ Roll No. _____
(For DSAS Personnel only) Attested by: <u>RODEL L. BALDADO, MHist, MAEd</u> OIC, Department of Student Affairs & Services Date: _____	

APPENDIX B Cont'd

51

APPENDICES

APPENDIX C: Letter for Species Identification

	Ozamiz City	
COLLEGE OF MEDICAL TECHNOLOGY		
CERTIFIED: ISO 21001: 2018 Educational Organizations Management System by DNV ACCREDITED: Philippine Association of Colleges and Universities Commission on Accreditation (PACUOCAO)		
January 7, 2026		
EDWARD B. YASAY		
Regional Director,		
Bureau of Fisheries and Aquatic Resources, Region 10		
Macaban, Cagayan de Oro City		
Subject: Request for species identification for the purpose of thesis		
Dear Sir Yasay,		
We, the undersigned 3rd-year Bachelor of Science in Medical Technology students from the College of Medical Technology, Misamis University, respectfully write to you regarding our research study entitled "Microbial and Heavy Metal Analysis of Blood Cockle (<i>Tegillarca granosa</i>, L.) in Panguil Bay, Philippines."		
The purpose of this letter is to formally request assistance in the species identification of the blood cockle samples collected for our scientific study, as accurate identification is essential to the validity of our research.		
This study is conducted solely for academic and research purposes, and all sample collection was carried out ethically and in accordance with established scientific standards. Should the Bureau of Fisheries and Aquatic Resources (BFAR) require a live specimen for proper identification, we would be willing to coordinate accordingly and may be contacted at your convenience.		
Your favorable consideration of this request will be highly appreciated. Thank you very much.		
Respectfully and with gratitude,		
 Lance Tristan D. Maghinay Researcher	 Karl Maxel D. Lao, RMT, MSc, MLS Research Adviser	
Noted by: 		
Evangeline M. Sinedo, RMT, MATMRS		
Dean, College of Medical Technology		
BFAR-10 / PFO-MIS. OCC.		
RECEIVED BY : <u>JC Apales</u>		
DATE : <u>1-7-2026</u>		
TIME : <u>3:56 PM</u>		

APPENDICES

APPENDIX D: Species Identification Result



RESULTS OF ANALYSIS

Source of Specimen : Ozamis City, Misamis Occidental
Submitted by : Lance Tristan D. Maghinay
Address : Ozamis City, Misamis Occidental
Date Submitted : January 23, 2026
Date of Completion of Analysis : January 28, 2026

Examination : Morphological Analysis
Kingdom : Animalia
Phylum : Mollusca
Class : Bivalvia
Order : Arcida
Family : Arcidae
Genus : *Tegillarca*
Species : *granosa*
Scientific Name : *Tegillarca granosa* (Linnaeus, 1758)
English name : Blood cockle
Local name : Litob



Figure 1. Specimen for morphological analysis of *Tegillarca granosa* (Linnaeus, 1758)

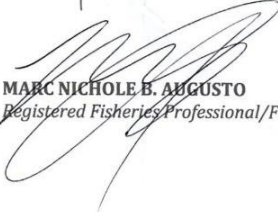
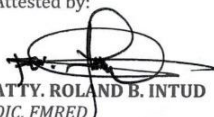
References:

Carpenter, K. E., & Niem, V. H. (Eds.). (1998). The living marine resources of the Western Central Pacific. Volume 1: Seaweeds, corals, bivalves and gastropods (FAO Species Identification Guide for Fishery Purposes). Food and Agriculture Organization of the United Nations.

WoRMS Editorial Board. (2025). *Tegillarca granosa* (Linnaeus, 1758). World Register of Marine Species.

APPENDICES

APPENDIX D Cont'd

 	Republika ng Pilipinas Department of Agriculture BUREAU OF FISHERIES AND AQUATIC RESOURCES - 10 Phone: (088) 856-9593 Makabalan, Cagayan de Oro City, Misamis Oriental 9000 Email: bfar10@bfar.da.gov.ph Website: https://r10.bfar.da.gov.ph Social Media: https://facebook.com/BFAR10 TIN No.: 000-550-823017	 BFAR - GENDER AND DEVELOPMENT
<u>CERTIFICATION</u>		
This is to certify that the samples submitted by Lance Tristan D. Maghinay and received by BFAR-10 last January 23, 2026, are species of scientifically known as <i>Tegillarca granosa</i> (Linnaeus, 1758) or locally known as "Litob".		
This certification is issued on the 28th of January 2026 at BFAR Regional Office, Cagayan de Oro City.		
Certified by:		
 PRINCESS MAE T. CENA <i>Marine Biologist/PARLC Member</i>		
 MARC NICHOLE B. AUGUSTO <i>Registered Fisheries Professional/Fish Examiner</i>		
Attested by:		
 ATTY. ROLAND B. INTUD <i>OIC, FMRED</i>		
Approved:		
 EDWARD B. YASAY <i>Regional Director</i>		
Page 2		
<i>Masaganang Agrikultura, Maunlad na Ekonomiya</i>		

APPENDICES

APPENDIX E: Documentation



E.1. Species Identification

APPENDICES
APPENDIX E Cont'd



E.2. Sample Collection and Processing(Homogenization and Serial Dilution)

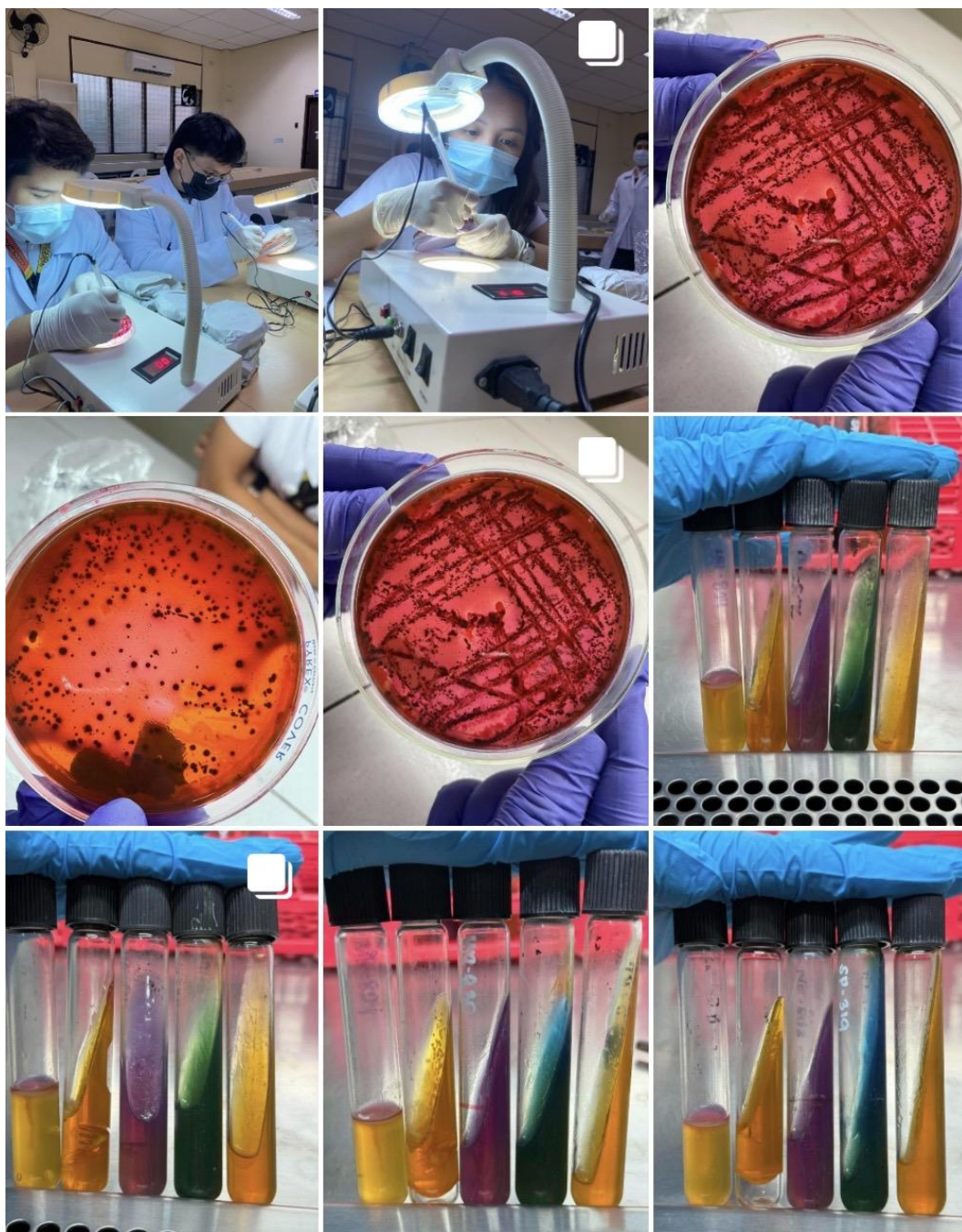
APPENDICES

APPENDIX E Cont'd



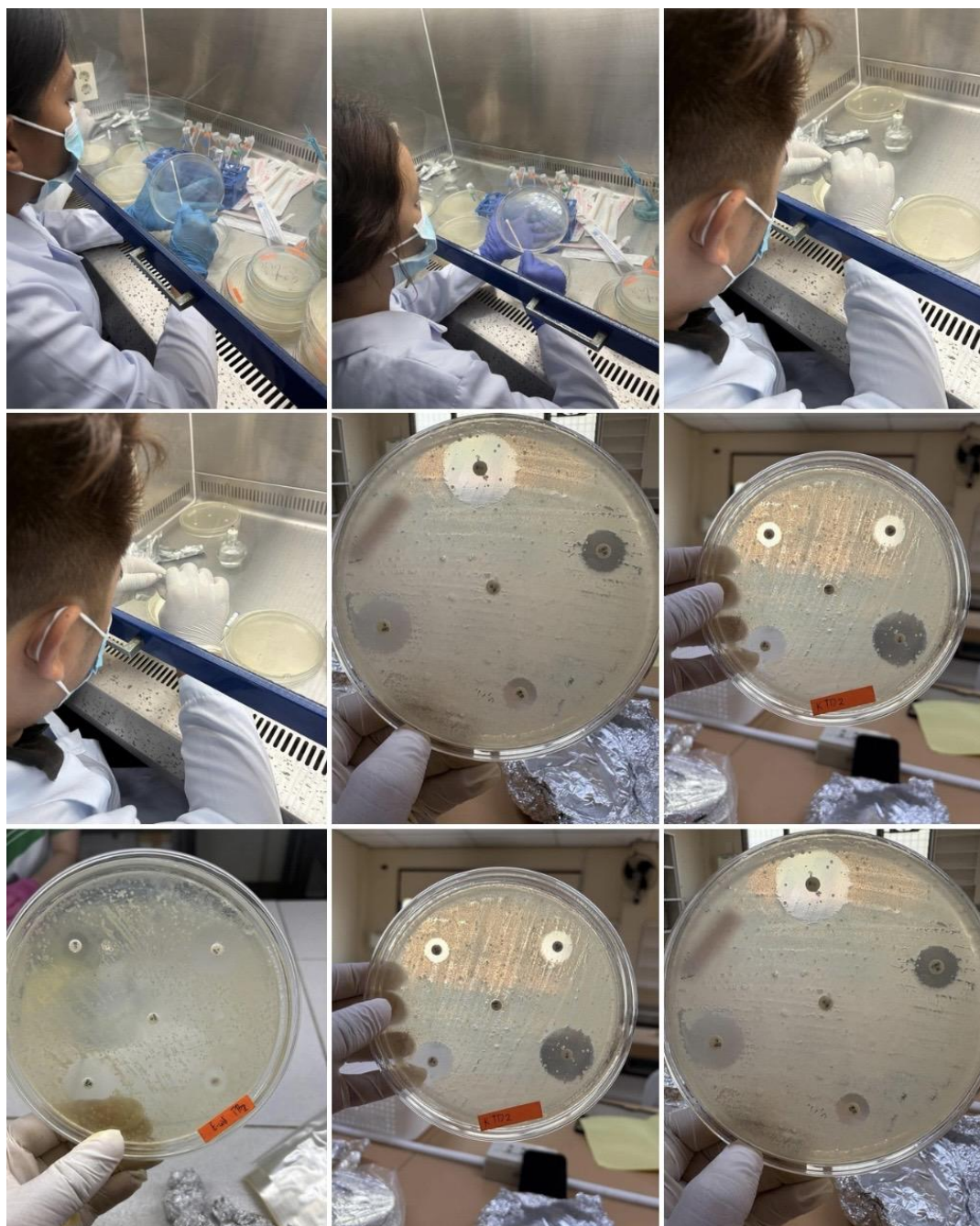
E.3. Presumptive, Confirmatory, and Confirmed

APPENDICES
APPENDIX E Cont'd



E.4. Colony Counting and Biochemical Test Result

APPENDICES
APPENDIX E Cont'd



E.5. Antibacterial Susceptibility Test against *Escherichia coli*

APPENDICES

APPENDIX F: Raw results

Sampling Site	Number of Colonies Counted
TANGUB	202
TANGUB	178
TANGUB	256
TANGUB	198
TANGUB	205
ETD2 AVERAGE = 277.4	
Sampling Site	Number of Colonies Counted
Baroy	285
Baroy	296
Baroy	279
Baroy	198
Baroy	229
ETD2 AVERAGE = 277.4	

F.1. Colony Counting Raw Results

APPENDICES

APPENDIX F Cont'd

Sample ID	Ampicillin	ciprofloxacin	Chloramphenicol	ceftriaxone	gentamicin
ETB2	0mm	11mm	3mm	17mm	4mm
ETB2	8mm	15mm	14mm	13mm	4mm
ETB2	13mm	25mm	26mm	23mm	14mm
ETB2	0mm	13mm	4mm	18mm	2mm
ETB2	0mm	11mm	2mm	20mm	4mm
ETB2	0mm	21mm	13mm	30mm	14mm
ETD2	8mm	14mm	17mm	11mm	12mm
ETD2	10mm	15mm	14mm	17mm	5mm
ETD2	10mm	15mm	14mm	24mm	8mm
ETD2	12mm	21mm	15mm	24mm	11mm
ETD2	0mm	24mm	21mm	29mm	11mm
ETD2	0mm	21mm	12mm	30mm	14mm

F.2 Antibiotic Susceptibility Testing- Zone of Inhibition

APPENDICES

APPENDIX F Cont'd



FOCUS ON EXCELLENCE • ADAPTABILITY TO CHANGE • SOCIAL RESPONSIBILITY • TEAMWORK • LEARNING AND GROWTH • ACCOUNTABILITY • BALANCED

F.A.S.T. Laboratories – CDO
Lapasan Hi-way corner Camp Alagar Road CDO City

Reference No. CD2603-1655
Page 1 of 2

TEST REPORT

CUSTOMER : JAMAICA L. ACAPULCO
ADDRESS : Brgy. 5, Tangub City
CONTACT PERSON : Jamaica L. Acapulco
CONTACT DETAILS : jamacapulco97@gmail.com
SAMPLE(S) SUBMITTED : SEA WATER (As Declared)
SAMPLE CODE : CD2603-1655-01
SAMPLED BY : Customer
DATE / TIME OF SAMPLING : 17 March 2026 / 8:25 PM
DATE / TIME RECEIVED : 18 March 2026 / 1:40 PM
ANALYZED BY : S.S. GABULE
DATE REPORTED : 30 March 2026

Parameter	Unit	Result	Test Method	Date and Time Analyzed
		Seawater 1 Tangub		
Nitrate as NO ₃ – N	mg/L	0.1	US EPA 352.1 Colorimetric, Brucine	20 Mar 2026 / 8:41 AM

Reference: United States Environmental Protection Agency (USEPA).

Results are those obtained at time of examination and relate only to the sample(s) tested.

CERTIFIED BY: *[Signature]*
M.R.T. SULLANO, RCh
Laboratory Supervisor
Chem. Reg. No. 0014490

APPROVED BY: *[Signature]*
M.L.S. MENDOZA, RCh
Laboratory Business Manager
Chem. Reg. No. 08239

Terms and Conditions:

1. Sampling details such as sample description, sampling location/point, date and time of sampling are customer declared information which can affect the validity of results and the laboratory cannot confirm its accuracy and therefore should not be held liable.
2. This report is prepared for the SOLE USE of the customer and is prepared based on the item submitted, the services required by the customer and the conditions under which the Services are performed by F.A.S.T. Laboratories. The report is not intended to be representative of similar or equivalent items and does not constitute an endorsement by F.A.S.T. Laboratories on the item.
3. The report shall not be reproduced EXCEPT IN FULL and SHALL NOT be used for advertisement, publicity material, and press release or for litigation purposes WITHOUT the written permission of F.A.S.T. Laboratories.
4. F.A.S.T. Laboratories shall under no circumstances be liable to the customer or its representatives for any direct or indirect loss or damage suffered by the customer or its representatives howsoever arising or whether connected with the services provided by FAST Laboratories
5. This report is NOT OFFICIAL and VALID unless stamped with the seal of F.A.S.T. Laboratories. It shall be kept on file for six (6) months from the date of issue.

ACCREDITATIONS/RECOGNITIONS: Dept. of Environment and Natural Resources (DENR) • Dept. of Health (DOH) • Dept. of Agriculture - Bureau of Animal Industry (DA-BAI) Food and Drug Administration (FDA) • Bureau of Fisheries and Aquatic Resources (BFAR) • Laguna Lake Development Authority (LLDA)

MAIN: 62, 20th Avenue, Cubao, Quezon City, 1109 • Tels. (02) 8912-0250- 8912-5275- Globe 0917-621-2625- www.fastlaboratories.com.ph • info@fastlaboratories.com.ph; mktgsales.fastlaboratories@gmail.com
BRANCHES: CALABARZON – B1 L2 P1 Ponte Verde de Sto. Tomas, Brgy. San Rafael, Sto. Tomas City, Batangas 4234 • Tels. (043)723-0424; 233-2787/Globe: (0917)-562-6049- mktgsales.fastlaboratories@gmail.com
Cebu – Hiway Central Bldg., M. C. Briones Highway, Mandaue City, Cebu 6014 • Tels. (032)-603-8272; Globe: (0917) 562-6322- E-mail: mktgsales.fastlaboratories@gmail.com
Cagayan de Oro – JF Castillo Bldg., C.M. Recto Ave., cor. Camp Alagar Rd., Brgy. Lapasan, Cagayan de Oro City, Misamis Oriental 9000 • Tels. (089)652-4946; (0917)624-6321- mktgsales.fastlaboratories@gmail.com
Clark – Angeles – Stall 5, 6, 7, SNB Bldg., 717 Magaling Road, Pampanga, Angeles City 2009 • Tels. (045)457-0517 • Globe: (0917) 108-8463- mktgsales.fastlaboratories@gmail.com

F.3. Nitrate Test Result From Water Sample (Sampling site: Tangub)

APPENDICES

APPENDIX F Cont'd



FOCUS ON EXCELLENCE • ADAPTABILITY TO CHANGE • SOCIAL RESPONSIBILITY • TEAMWORK • LEARNING AND GROWTH • ACCOUNTABILITY • BALANCED

F.A.S.T. Laboratories – CDO
Lapasan Hi-way corner Camp Alagar Road CDO City

Reference No. CD2603-1655
Page 2 of 2

TEST REPORT

CUSTOMER : JAMAICA L. ACAPULCO
ADDRESS : Brgy. 5, Tangub City
CONTACT PERSON : Jamaica L. Acapulco
CONTACT DETAILS : jamacapulco97@gmail.com
SAMPLE(S) SUBMITTED : SEA WATER (As Declared)
SAMPLE CODE : CD2603-1655-02
SAMPLED BY : Customer
DATE / TIME OF SAMPLING : 17 March 2026 / 8:38 PM
DATE / TIME RECEIVED : 18 March 2026 / 1:40 PM
ANALYZED BY : S.S. GABULE
DATE REPORTED : 30 March 2026

Parameter	Unit	Result	Test Method	Date and Time Analyzed
		Seawater 2 Tubod		
Nitrate as NO ₃ – N	mg/L	0.1	US EPA 352.1 Colorimetric, Brucine	20 Mar 2026 / 8:41 AM

Reference: United States Environmental Protection Agency (USEPA).

Results are those obtained at time of examination and relate only to the sample(s) tested.

CERTIFIED BY:
M.R.T. SULLANO, RCh
Laboratory Supervisor
Chem. Reg. No. 0014490

APPROVED BY:
M.L.S. MENDOZA, RCh
Laboratory Business Manager
Chem. Reg. No. 08239

Terms and Conditions:

1. Sampling details such as sample description, sampling location/point, date and time of sampling are customer declared information which can affect the validity of results and the laboratory cannot confirm its accuracy and therefore should not be held liable.
2. This report is prepared for the SOLE USE of the customer and is prepared based on the item submitted, the services required by the customer and the conditions under which the Services are performed by F.A.S.T Laboratories. The report is not intended to be representative of similar or equivalent items and does not constitute an endorsement by F.A.S.T Laboratories on the item.
3. The report shall not be reproduced EXCEPT IN FULL and SHALL NOT be used for advertisement, publicity material, and press release or for litigation purposes WITHOUT the written permission of F.A.S.T Laboratories.
4. F.A.S.T Laboratories shall under no circumstances be liable to the customer or its representatives for any direct or indirect loss or damage suffered by the customer or its representatives howsoever arising or whether connected with the services provided by FAST Laboratories
5. This report is NOT OFFICIAL and VALID unless stamped with the seal of F.A.S.T Laboratories. It shall be kept on file for six (6) months from the date of issue.

ACCREDITATIONS/RECOGNITIONS: Dept. of Environment and Natural Resources (DENR) • Dept. of Health (DOH) • Dept. of Agriculture - Bureau of Animal Industry (DA-BAI) Food and Drug Administration (FDA) • Bureau of Fisheries and Aquatic Resources (BFAR) • Laguna Lake Development Authority (LLDA)

MAIN: 62, 20th Avenue, Cubao, Quezon City, 1109 • Tels. (02) 8912-0250- 8912-5275 • Globe 0917-621-2625 • www.fastlaboratories.com.ph • info@fastlaboratories.com.ph; mktgsales.fastlaboratories@gmail.com
BRANCHES: CALABARZON - B1 L2 P1 Ponto Verde de Sto. Tomas, Brgy. San Rafael, Sto. Tomas City, Batangas 4234 • Tels. (043) 723-0424, 233-2797/Globe: (0917) 562-6049 • mktgsales.fastlaboratories@gmail.com
Cebu - Highway Central Bldg. M. C. Birones Highway, Mandaua City, Cebu 6014 • Tels. (032) 503-8272, Globe: (0917) 562-6322 • E-mail: mktgsales.fastlaboratories@gmail.com
Cagayan de Oro - 2/F Casillo Bldg., C.M. Rendo Ave., cor Camp Alagar Rd., Brgy. Lapasan, Cagayan de Oro City, Misamis Oriental 9000 • Tels. (089) 952-4542, (0917) 524-5321 • mktgsales.fastlaboratories@gmail.com
Clark - Angeles - Stall 5,6,7, SNB Bldg., 717 Magalang Road, Pandan, Angeles City 2009 • Tels. (045) 457-0517 • Globe: (0917) 106-6463 • mktgsales.fastlaboratories@gmail.com

F.4. Nitrate Test Result From Water Sample (Sampling site: Baroy)

APPENDICES

APPENDIX F Cont'd



FOCUS ON EXCELLENCE • ADAPTABILITY TO CHANGE • SOCIAL RESPONSIBILITY • TEAMWORK • LEARNING AND GROWTH • ACCOUNTABILITY • BALANCED

F.A.S.T. Laboratories – CDO
Lapasan Hi-way corner Camp Alagar Road CDO City

Reference No. CD2603-1656
Page 1 of 1

TEST REPORT

CUSTOMER : JAMAICA L. ACAPULCO
ADDRESS : Brgy. 5, Tangub City
CONTACT PERSON : Jamaica L. Acapulco
CONTACT DETAILS : Jamacapulco97@gmail.com
SAMPLE(S) SUBMITTED : BLOOD COCKLE (In Ziplock) (As Declared)
SAMPLE CODE : CD2603-1656-01 to -02
SAMPLED BY : Customer
DATE / TIME RECEIVED : 18 March 2026 / 1:40 PM
DATE ANALYZED : 26 March – 07 April 2026
ANALYZED BY : J.A. CANATOY
DATE REPORTED : 07 April 2026

Sample(s) as Received	Results	Test Method
	Arsenic, mg/kg	
Blood Cockle 1 (Litub) – Tangub (CD2603-1656-01)	Less than 0.05**	Silver Diethyldithiocarbamate- Colorimetric Method (Modified)
Blood Cockle 2 (Litub) – Tubod (CD2603-1656-02)	Less than 0.05**	

Note: **-Reporting Limit

Reference: Official Method of Analysis of AOAC International, 21st ed., 2019.
Food Chemical Codex, 5th Edition.

Results are those obtained at time of examination and relate only to the sample(s) tested.

CERTIFIED BY: *[Signature]*
M.R.T. SULLANO, RCh
Laboratory Supervisor
Chem. Reg. No. 0014490

APPROVED BY: *[Signature]*
M.L.S. MENDOZA, RCh
Laboratory Business Manager
Chem. Reg. No. 08239

Terms and Conditions:

1. Sampling details such as sample description, sampling location/point, date and time of sampling are customer declared information which can affect the validity of results and the laboratory cannot confirm its accuracy and therefore should not be held liable.
2. This report is prepared for the **SOLE USE** of the customer and is prepared based on the item submitted, the services required by the customer and the conditions under which the Services are performed by F.A.S.T. Laboratories. The report is not intended to be representative of similar or equivalent items and does not constitute an endorsement by F.A.S.T. Laboratories on the item.
3. The report shall not be reproduced **EXCEPT IN FULL** and **SHALL NOT** be used for advertisement, publicity material, and press release or for litigation purposes **WITHOUT** the written permission of F.A.S.T. Laboratories.
4. F.A.S.T. Laboratories shall under no circumstances be liable to the customer or its representatives for any direct or indirect loss or damage suffered by the customer or its representatives howsoever arising or whether connected with the services provided by FAST Laboratories
5. This report is **NOT OFFICIAL** and **VALID** unless stamped with the seal of F.A.S.T. Laboratories. It shall be kept on file for six (6) months from the date of issue.

ACCREDITATIONS/RECOGNITIONS: Dept. of Environment and Natural Resources (DENR) • Dept. of Health (DOH) • Dept. of Agriculture - Bureau of Animal Industry (DA-BAI)
Food and Drug Administration (FDA) • Bureau of Fisheries and Aquatic Resources (BFAR) • Laguna Lake Development Authority (LLDA)
MAIN: 62, 20th Avenue, Cubao, Quezon City, 1109 • Tels: (02) 8912-0250-8912-5275 • Globe 0917-621-2625 • www.fastlaboratories.com.ph • info@fastlaboratories.com.ph • mktg@fastlaboratories.com.ph
BRANCHES: CALABARZON – 9112 P/P Ponto Verde de Sto. Tomas, Brgy. San Rafael, Sto. Tomas City, Batangas 4234 • Tels: (043) 723-0424; 233-27973000; (0917) 562-6249 • mktg@fastlaboratories.com.ph
Cebu – Hi-way Central Bldg. M. C. Briones Highway, Mandaue City, Cebu 6014 • Tels: (032) 503-8272; Globe: (0917) 562-6322 • E-mail: mktg@fastlaboratories.com.ph
Cagayan de Oro – 2nd Casillo Bldg. C.M. Rocio Ave., cor. Camp Alagar Rd., Brgy. Lapasan, Cagayan de Oro City, Misamis Oriental 9000 • Tels: (088) 852-4846; (0917) 624-6321 • mktg@fastlaboratories.com.ph
Clark – Angeles – 5th 5.6.7, 5th Bldg., 717 Magalang Road, Pandan, Angeles City 2009 • Tels: (045) 457-0617 • Globe: (0917) 108-8463 • mktg@fastlaboratories.com.ph

F.5. Heavy Metal Analysis Result from Bivalve Flesh(*Tegillarca granosa*.,L) from sample collected from Tangub and Baroy

APPENDICES

APPENDIX G: Turnitin and Grammarly results



Page 2 of 59 - Integrity Overview

Submission ID trn:oid::28447:143938477

21% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

Filtered from the Report

▸ Bibliography

Match Groups

-  **129 Not Cited or Quoted 17%**
Matches with neither in-text citation nor quotation marks
-  **31 Missing Quotations 4%**
Matches that are still very similar to source material
-  **2 Missing Citation 0%**
Matches that have quotation marks, but no in-text citation
-  **0 Cited and Quoted 0%**
Matches with in-text citation present, but no quotation marks

Top Sources

- 11%  Internet sources
- 9%  Publications
- 18%  Submitted works (Student Papers)

Integrity Flags

1 Integrity Flag for Review

-  **Hidden Text**
17 suspect characters on 2 pages
Text is altered to blend into the white background of the document.

Our system's algorithms look deeply at a document for any inconsistencies that would set it apart from a normal submission. If we notice something strange, we flag it for you to review.

A Flag is not necessarily an indicator of a problem. However, we'd recommend you focus your attention there for further review.



Page 2 of 59 - Integrity Overview

Submission ID trn:oid::28447:143938477

APPENDICES

APPENDIX G Cont'd

Report: MICROBIAL CONTAMINATION, RESISTANCE, AND HEAVY METAL ANALYSIS OF BLOOD COCKLE (Tegillar...

MICROBIAL CONTAMINATION, RESISTANCE, AND HEAVY METAL ANALYSIS OF BLOOD COCKLE (Tegillarca granosa, L.) IN PANGUIL BAY, PHILIPPINES

by Misamis University

General metrics

64,018	8,603	711	34 min 24 sec	1 hr 6 min
characters	words	sentences	reading time	speaking time

Score



This text scores better than 99%
of all texts checked by Grammarly

Writing Issues

7	3	4
Issues left	Critical	Advanced

Plagiarism

This text hasn't been checked for plagiarism

CURRICULUM VITAE

PERSONAL INFORMATION

Name : Jamaica L. Acapulco
Age : 21
Birthdate : November 28, 2004
Civil Status : Single
Nationality : Filipino
Home Address : P - 2 Brgy. 5, Tangub City Misamis Occidental
Current Address : P - 2 Brgy. 5, Tangub City Misamis Occidental
Contact Number : 09279865273
E-mail Address : jamacapulco97@gmail.com

EDUCATIONAL BACKGROUND

TERTIARY : Bachelor of Science in Medical Technology
Misamis University
2024 - present

Secondary : Senior High School
Science, Technology, Engineering, and Mathematics
Tangub City National High School
2021-2023

Junior High School
Tangub City National High School
2017 - 2020

Elementary : Tangub City Central School
2011-2017

CURRICULUM VITAE

PERSONAL INFORMATION

Name : Angie Lee G. Lungay
Age : 20
Birthdate : May 26,2005
Civil Status : Single
Nationality : Filipino
Home Address : P-4 Banglay, Tangub City, Misamis Occidental
Current Address : P-4 Banglay, Tangub City, Misamis Occidental
Contact Number : 09663110406
E-mail Address : lungayangie2005@gmail.com

EDUCATIONAL BACKGROUND

TERTIARY : Bachelor of Science in Medical Technology
Misamis University
2023- present

Secondary : Senior High School
Northwestern Mindanao State College of Science and
Technology
2021-2023

Junior High School
Banglay National High School
2017-2020

Elementary : Banglay Elementary School
Tangub City
2011-2017

CURRICULUM VITAE

PERSONAL INFORMATION

Name : Lance Tristan D. Maghinay
Age : 21
Birthdate : February 03, 2005
Civil Status : Single
Nationality : Filipino
Home Address : P-8 Aguada, Ozamiz City, Misamis Occidental
Current Address : P-8 Aguada, Ozamiz City, Misamis Occidental
Contact Number : 09292949667
E-mail Address : Lancetristan5@gmail.com

EDUCATIONAL BACKGROUND

TERTIARY : Bachelor of Science in Medical Technology
Misamis University
2026- present

Secondary : Senior High School
Science, Technology, Engineering, and Mathematics
Lycée St. Jean Baptiste de La Salle
2021-2023

Junior High School
Dr. Cecilio Putong National High School (Bohol National
High School)
Tagbilaran City, Bohol
2018-2020

Jagobiao National High School
Mandaue, Cebu City
2017-2018

Elementary : Catadman Elementary School
Ozamiz City
2011-2017

CURRICULUM VITAE

PERSONAL INFORMATION

Name : Aaron Charles L. Opada
Age : 22
Birthdate : November 4, 2003
Civil Status : Single
Nationality : Filipino
Home Address : Baroy, Iligan City, Lanao Del Norte
Current Address : Lam-an, Ozamis City, Misamis Occidental
Contact Number : 09815340026
E-mail Address : opadaaaroncharles@gmail.com

EDUCATIONAL BACKGROUND

TERTIARY : Bachelor of Science in Medical Technology
Misamis University
2024 - present

Bachelor of Science in Medical Technology
Adventist Medical Center College
2022 - 2024

Secondary : Senior High School
Humanities and Social Science
Saint Michael's College, Iligan City
2020 - 2022

Junior High School
Saint Thomas Aquinas School Montessori
2018 - 2020

Corpus Christi Parochial School of Iligan
2016 - 2018

Elementary : Corpus Christi Parochial School of Iligan
2010-2016

CURRICULUM VITAE

PERSONAL INFORMATION

Name : Glyka Crizan Mae B. Pilapil
Age : 21
Birthdate : Pangabuan, Tangub City
Civil Status : Single
Nationality : Filipino
Home Address : P-2 Pangabuan, Tangub City, Misamis Occidental
Current Address : P-2 Pangabuan, Tangub City, Misamis Occidental
Contact Number : 09567502362
E-mail Address : crznjane@gmail.com

EDUCATIONAL BACKGROUND

TERTIARY : Bachelor of Science in Medical Technology
Misamis University
2026- present

Secondary : Senior High School
Science, Technology, Engineering, and Mathematics
St. Michael's High School
2021-2023

Junior High School
St. Michael's High School
2017-2020

Elementary : Pangabuan Elementary School
Tangub City
2011-2017