Research Article

Selected Mollusks from Pujada Bay, Philippines: Heavy Metal Health Risk Assessment and Antibacterial Activities

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ABSTRACT

The study assessed the human health risks of heavy metals and the antibacterial activities of commonly consumed mollusk species, *Anadara maculosa, Antigona puerpera, Canarium urceus* and *Lambis lambis* from Guang-guang, Pujada Bay, City of Mati, Davao Oriental, Philippines. Cadmium (Cd) and lead (Pb) contents were determined using Atomic Absorption Spectrophotometer. On the other hand, the agar well diffusion method was used for the antibacterial activity determination. Water quality, which includes temperature, pH, dissolved oxygen and total dissolved solids, were also investigated. The Cd and Pb concentrations in four marine mollusks were below the standard limit for Cd and Pb in fish and fishery/aquatic products set by the FAO No.210 series of 2001. Furthermore, the result of the human health risk assessment using Chronic Daily Intake, Hazard Quotient and carcinogenic risk for Cd and Pb were below the acceptable level set by the United States Environmental Protection Agency. Cd and Pb metals uptake through marine mollusc ingestion posed no potential non-carcinogenic and carcinogenic risks to human health. On the other hand, the results of the physico-chemical parameters of seawater were within the standard limits set by DAO No. 2016-08 Water Quality Guidelines and General Effluent Standards of 2016, indicating that Guang-guang, Pujada Bay has a good marine water condition. Moreover, only *C. urceus* crude extract showed antibacterial activity against *Staphylococcus aureus* bacteria. Hence, further studies on the content of its natural products are highly recommended.

Key words: Antibacterial activity, Heavy metals, Mollusks, Risk Assessment, Cadmium, Lead.

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INTRODUCTION

The marine ecosystem is the home of various kinds of marine organisms that serve as the source of food and livelihood of the community. The marine waters provide fishing, swimming, shellfish harvesting and many other recreational activities of the coastal communities. However, its importance to humans is being threatened by various pressures in the environment.[1] Pollution in water bodies had become a significant problem in many parts of the world due to both natural and anthropogenic processes. According to the United States Environmental Protection Agency,[2] the increased coastal development, pollution from ships, land-based sources of pollution, destruction of habitat and other threats to marine waters are the reasons why the world's coastal waters and oceans are deteriorating. Most of the chemicals used in industries, agricultural lands and domestic wastes are disposed of in water channels that all eventually transported to ocean waters.[3] These various human activities may be the cause of heavy metal enrichment in sediments and water that affects marine organisms' health, diversity and abundance.[4]

The existence of toxc chemicals or heavy metals in the aquatic ecosystem poses serious health threats to many living organisms, including humans. Heavy metal concentrations in an aquatic ecosystem can cause carcinogenic, reproductive and developmental effects

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on marine organisms.[5] On the other hand, human exposure to heavy metals even in lower concentrations can pose different health diseases[6] and may lead to carcinogenic or non-carcinogenic health risks.[7]

Marine invertebrates such as molluscs and crustaceans are known to accumulate heavy metals in marine waters and sediments.[8] Bivalves and gastropods are the types of molluscs commonly used in bio monitoring of aquatic pollution. These molluscs are bottom dwellers and filter-feeders. They primarily feed on phytoplankton, inhale water with oxygen and absorb food particles through their gills. Due to their feeding mechanisms, they are susceptible to different contaminants present in their environment.[9] However, marine organisms such as mollusc are found to contain natural products and can become a potential source of novel pharmaceutical compounds for anticancer, antioxidant, antibiotic and among others.[10]

Several studies have been conducted on the different aquatic resources existing in Pujada Bay, including environmental monitoring of contaminants in sediments, water and marine organisms. However, no study has been reported on the human health risk of heavy metals (cadmium and lead) and the antibacterial properties of four selected mollusk species namely; *Anadara maculosa, Antigona puerpera, Canarium urceus* and *Lambis lambis* in Guang-guang, Pujada Bay, City of Mati, Davao Oriental. Hence, this study.

MATERIALS AND METHODS

Study area

Guang-guang is one of the coastal water areas situated along the Pujada Bay. (Figure 1) which is declared as Protected Landscape and Seascape on July 31, 1994, under the Presidential Proclamation No. 431. Pujada Bay is located in Southern Mindanao between the GPS coordinates of 6°48'04" and 6°54'25" N latitude and between 126°9'08" and 126°19'33" E longitude. It has an area of approximately 21,200 hectares, lying on the southeastern part of Mindanao. It is established and reserved for the protection and conservation of the importance of the marine ecosystem.[11] Diverse species of sea grasses, mangroves, fishes, macro benthos and other forms of marine organisms are present in its vicinity.

Sample collection, preparation and heavy metal analysis

Marine mollusk samples

Four (4) types of marine mollusk species were randomly handpicked and collected in Guang-guang

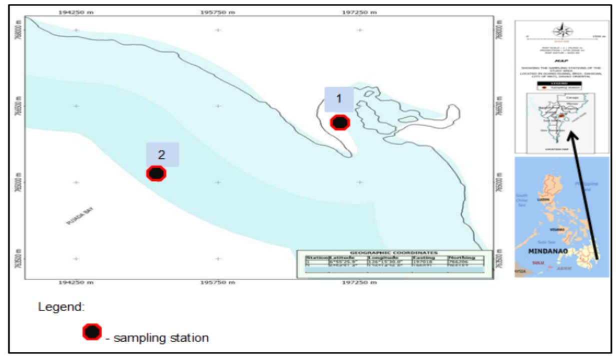

Figure 1: Map showing the sampling stations of the study area in Guang-guang, Barangay Dahican, City of Mati, Davao Oriental (CENRO, 2017).

(Figure 2). These were: *A. maculosa*, *A. puerpera*, *C. urceus* and *L. lambis*. The identification of the species was authenticated by a marine biologist and the director of the Regional Integrated Coastal Resource Management Center (ICRM). The Regional ICRM Center (RIC-XI) is the sole center on the island of Mindanao, Philippines. The collected bivalves and gastropods mollusk species were properly cleaned with distilled water. All mollusk samples were blanched and its soft tissues were removed from the shells and air-dried. One gram of dried, ground powdered sample was weighed accurately in a porcelain crucible. It was placed in a muffle furnace at 500°C for 2 hr or until the ash became grayish white. The cooled ash was moistened with 1 mL distilled water and then added with three to four mL of concentrated HNO_3. The mixture was heated on a hot plate at 100 to 120°C to evaporate excess nitric acid. It was then transferred into a 50 mL volumetric flask and diluted to mark with distilled water. The solution was used for the determination of heavy metals (Cd and Pb) using the Atomic Absorption Spectrophotometer based on the Official Methods of Analysis of AOAC International, 19th edition.[12]

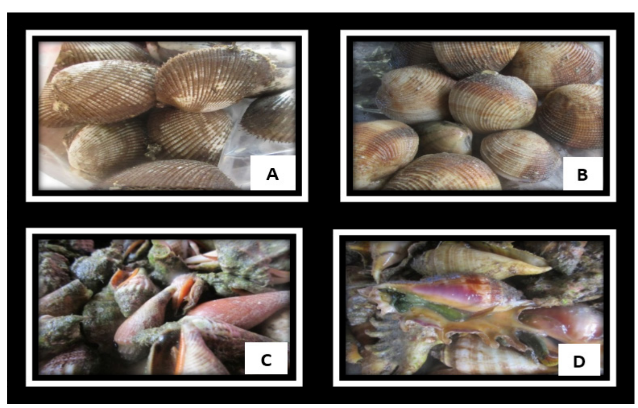

Figure 2: Selected mollusks collected from Guang-guang, Pujada Bay, Davao Oriental, Philippines: A. *A. maculosa* (Reeve, 1884), B. *A. puerpera* (Linnaeus, 1771), C. *C. urceus,* (Linnaeus, 1758), D. *L. lambis* (Linnaeus, 1758).

Water samples

On the other hand, 4-L composite seawater samples were also collected to determine the physico-chemical parameters of water in two sampling stations in Guang-guang, Pujada Bay. These include the pH, dissolved oxygen and total dissolved solids of the water. The methods used followed the Standard Methods for the Examination of Water and Wastewater, 22nd edition.[13]

Antibacterial activities

About 20 g of each dried samples was treated with 95% ethanol for 24 to 48 hr. The mixture was centrifuged and filtered and the residue was washed with fresh portions of alcohol. The filtrate was then concentrated under *vacuo* at a temperature below 50°C using a rotary evaporator. The crude extract was stored in a tightly closed vial at 0 to 5°C until its analysis.

The ethanolic crude extract of molluscs samples was subjected to antimicrobial test against pathogenic gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* using agar well diffusion method. Ampicillin and penicillin were used as standard drugs, respectively, for studying the activities of the extract.

The melted agar was poured into dry sterile petri dishes and solidified. A sterile cotton swab moistened with the test suspension that was previously incubated in Muller-Hinton Broth for 24 hr at 35°C was used to steak over the entire surface of the agar. It was allowed to stand for 5 min. A cork borer was sterilized by immersing it in an erlenmeyer flask containing ethanol and heated over an alcohol flame. The agar was stabbed using the cooled cork borer to the bottom of the dish to create a well. The well was filled with the previously prepared crude ethanol extract of mollusc samples. The incubation period was 24 hr at 35°C. The extract's activity was determined by measuring the diameter of the zone of inhibition and compared with the values produced from the standard drugs.

Data analysis

All results were expressed in terms of mean and standard deviation. Data were further processed to estimate the risk analysis based on available standards.

Risk quotient

$$RQ = \frac{\text{(Monitoring concentration of i)}}{\text{(Allowable concentration of i)}} \quad (1)$$

Where (i) is the monitored concentration of pollutants. The allowable concentration of pollutants (i) is recommended by USEPA and other international organizations.

Human health risk Indices

Hazard Quotient

Human health risk for heavy metals potential non-carcinogenic effects was obtained by comparing the calculated contaminant from the exposure route (ingestion) with the reference dose (RfD) to develop hazard quotient (HQ) which was determined using equation 2:

$$HQ_{ing} = \frac{Exp_{ing}}{RfD_{ing}} \quad (2)$$

$$Exp_{ing} = \frac{C \times IR \times EF \times ED}{EW \times AT} \quad (3)$$

Where,

HQ_{ing} hazard quotient via ingestion (unitless).

Exp_{ing} exposure to heavy metals through ingestion.

RfD_{ing} oral reference dose (µg/kg/d).

The exposure doses were calculated using Navededullah *et al*. 2014.[14] The value of exposure factors and their respective units are shown in Table 1.

Chronic daily intake

Chronic daily intake (CDI) is the amount of chemical to which a person can be exposed each day for a longer period without suffering harmful effects (mg/kg/day). CDI is calculated using equation 4:

$$CDI = Cx \frac{DI}{BW} \quad (4)$$

Where C represents the concentration of heavy metal in marine mollusk (mg/kg), DI for the average daily intake rate (mg/kg/day) and BW for the bodyweight (70kg), respectively.

Carcinogenic risk

In order to show the lifetime carcinogenic risk (CR) of heavy metal ingestion, equation 5 is used:

$$CRI_{ing} = \frac{Exp_{ing}}{SF_{ing}} \quad (5)$$

Where,

CR_{ing} - values of carcinogenic risk via ingestion route (unit less);

Exp_{ing} - is the carcinogenic risk through ingestion route $(\mu g/g/d)^{-1}$

SF_{ing} - is the carcinogenic slope factor, ingestion $(\mu g/g/d)^{-1}$.

Table 1: Exposure factors, its corresponding unit and values (Naveedullah *et al.* 2014).

Exposure factor	Symbol	Unit	Values
Concentration of Cd or Pb in mollusk	$C_{mollusk}$	µg/g or mg/Kg	
Mollusk ingestion rate/ Average daily dose	IR	Kg/day/ person	0.082
Exposure Frequency	EF	day/year	360
Exposure Duration	ED	Year	30
Average body weight	BW	Kg	70
Averaging Time	AT	Days	10,950
Oral reference dose			
Cd		mg/Kg/day	0.001
Pb			0.004
Slope Factor			
Cd	SF	$(\mu g/g/d)^{-1}$	6.1×10^3
Pb			8.5

RESULTS

Levels of heavy metals in the marine mollusk

The concentration of Cd and Pb for the molluscs samples are shown in Figure 3. Cd concentrations for both bivalves, *A. maculosa* and *A. puerpera,* were 0.03 µg/g. For the gastropods, Cd concentration was 0.06 µg/g in *C. urceus* while 0.08 µg/g in *L. lambis*. These values were below the standard limit for Cd (0.5 µg/g), as set by Fisheries Administrative Order (FAO) No. 210.[15] On the other hand, Pb concentrations in both bivalves and gastropod mollusks were all equal (0.10 µg/g) and below the standard level for Pb (0.5 µg/g) set by FAO No. 210[15] and other international organizations.

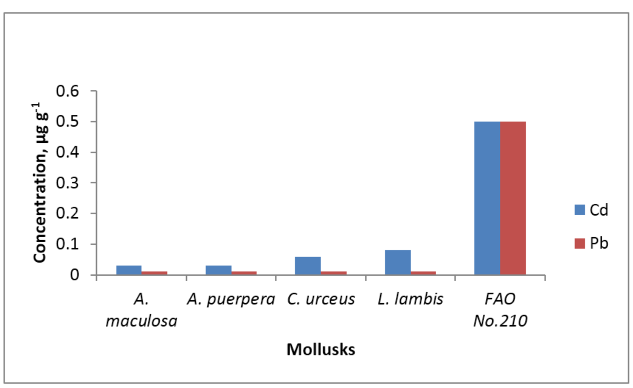

Figure 3: Concentrations of heavy metals (Cd and Pb) in marine mollusks soft tissues (µg/g).

Risk Assessment Indices

In assessing the potential adverse impact of heavy metals on the aquatic ecosystem, Risk quotient, or RQs were calculated and are shown in Table 2. RQ values for Cd were 0.06 µg/g for both *A. maculosa* and *A. puerpera*, 0.12 µg/g for *C. urceus* and 0.16 µg/g for *L. lambis*. On the other hand, RQs for Pb was 0.2 µg/g in all molluscs sample.

Human health risk assessment indices such as *HQ, CDI* and CR_{ing} were summarized in Table 3. HQ was applied to evaluate the non-carcinogenic health risk of marine mollusc ingestion using the exposure values. The values measured for Cd in both bivalve molluscs (*A. maculosa* and *A. puerpera*) was 0.035 while in gastropod molluscs, *C. urceus* had an 0.069 and 0.092 in *L. lambis*. On the other hand, values for Pb in all marine mollusk samples (*A. maculosa* and *A. puerpera, C. urceus* and *L. lambis*) were 0.029.

The obtained CDI value of Cd was 3.51×10^{-5} in both bivalve, *A. maculosa* and *A. puerpera* while in gastropods, 7.03×10^{-5} in *C. urceus* and 9.37×10^{-5} in *L. lambis*. On the other hand, the calculated CDI values of Pb in all marine mollusk samples were 1.17×10^{-4}. Thus, the of Pb from the four marine mollusks were slightly higher compared to the of Cd.

Carcinogenic risks of heavy metals through marine mollusc consumption were evaluated to determine whether a certain chemical probably pose cancer risks or not, to humans. The calculated CR_{ing} of Cd in both bivalve's molluscs *A. maculosa* and *A. puerpera* were 5.68×10^{-9} whereas, CR_{ing} of Cd in gastropods molluscs, *C. urceus* and *L. lambis* were 1.14×10^{-8} and 1.52×10^{-8}, respectively. These values were less than the standard range of carcinogenic risk factor of 1.0×10^{-6} to 1.0×10^{-4}.[14] On the other hand, the of Pb in all the mollusk species (1.36×10^{-5}) was still within the permissible range.

Physico-chemical characteristics of seawater

Figure 4 shows the water quality based on the parameters tested against the standard set by the Department of Environment and Natural Resources (DENR) Administrative Order (DAO) No. 2016-08. The temperature recorded during the collection of mollusc and water samples was 30°C in both stations 1 and 2, while pH had a range of 6.5 to 9. Dissolved oxygen levels, on the other hand, in both sampling stations were 7.7 mg/L. The total dissolved solids of 35,646 mg/L in station 1 was slightly higher than in station 2 (35,616 mg/L).

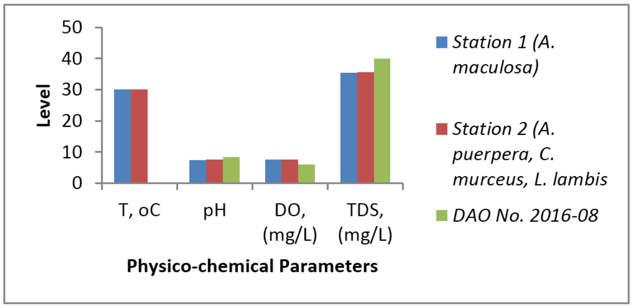

Figure 4: Physico-chemical characteristics of seawater in established Sampling sites.
Antibacterial activity

Antibacterial Activity

The mollusc extracts using ethanol solvent showed different values of the zone of inhibition against *E. coli* and *S. aureus*, as shown in Table 4. Among the four molluscs, it was *C. urceus* that showed activity against *S. aureus* bacteria with a 10.78 mm zone of inhibition. However, this value is weaker compared to the 30.44 mm zone of inhibition exhibited by the standard ampicillin drug.

Table 2: Risk Quotients of Cd and Pb metals.

Heavy Metals	Standard levels (FAO No. 210 series of 2001)	Unit	Risk Quotients: Bivalves		Gastropods		Remarks
			A. maculosa	*A. puerpera*	*C. urceus*	*L. lambis*	
Cd	0.5	ppm/ mg/ kg/ µg/g	0.06	0.06	0.12	0.16	RQ values (RQ<1) of both Cd and Pb metals posed no effects on marine organisms
Pb	0.5	ppm/ mg/ kg/ µg/g	0.2	0.2	0.2	0.2	

Table 3: Adult exposure to heavy metals through marine mollusk ingestion.

Bivalves			HQ		CDI (mg/kg/day)		CR_{ing}	
	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb
A. maculosa	3.47×10^{-5}	1.16×10^{-5}	0.035	0.029	3.51×10^{-5}	1.17×10^{-4}	5.68×10^{-9}	1.36×10^{-5}
A. puerpera	3.47×10^{-5}	1.16×10^{-5}	0.035	0.029	3.51×10^{-5}	1.17×10^{-4}	5.68×10^{-9}	1.36×10^{-5}
Gastropods								
C. urceus	6.93×10^{-5}	1.16×10^{-5}	0.069	0.029	7.03×10^{-5}	1.17×10^{-4}	1.14×10^{-8}	1.36×10^{-5}
L. lambis	9.24×10^{-5}	1.16×10^{-5}	0.092	0.029	9.37×10^{-5}	1.17×10^{-4}	1.52×10^{-8}	1.36×10^{-5}
US EPA			HQ<1				1×10^{-6} to 1×10^{-4}	

Table 4: Antimicrobial activity of selected mollusk.

Micro-organism	Zone of Inhibition, mm: *A. maculosa*	*A. puerpera*	*C. urceus*	*L. lambis*
S. aureus	6.00 30.33*	6.00 30.67*	10.78 30.44*	6.00 30.33
E. coli	6.00 31.67*	6.00 31.33*	6.00 31.67*	6.00 31.33

*Average value for the positive control, ampicillin (for *S. aureus*) and penicillin (for *E. coli*), respectively.

DISCUSSION

Levels of heavy metals in the marine mollusk

The observed differences between gastropods and bivalves in accumulating Cd heavy metal in this study were probably due to the type of mollusc, their feeding abilities, position in the water column, sedentary habits and other factors.[9-18] Gastropods burrowed on sediments using their radula in obtaining food while bivalves use their gills in taking food particles in the seabed.[19] Furthermore, gastropods are more developed and their ability to accumulate heavy metals is different from bivalves. Gastropods have well-developed heads with tentacles and asymmetrical body. Moreover, most of the gastropods are burrowers and some are herbivores, detritus feeders, predatory carnivores, scavengers, parasites and also a few are biliary feeders. On the other hand, bivalves have no tentacles and radula. It has either a rudimentary head or no head at all. Bivalves are mostly particulate, or filter feeders and accumulate organic matter.[20] However, the low concentration of Cd and Pb in both bivalves and gastropod molluscs is an indication of low levels of such heavy metals in the marine ecosystem of Guang-guang, Pujada Bay. Human activities in the Bay, such as wastewater discharge may not contain high amounts of heavy metals such as Cd and Pb.

Risk assessment Indices

RQs of Cd and Pb were all less than the permissible level of 1. RQ<1 means that the potential adverse effects to marine organisms caused by pollutant exposure are minimum, whereas RQ>1 means the potential adverse effects to marine organisms caused by pollutant exposure may be severe.[21]

Humans are exposed to heavy metals in different exposure routes (ingestion, dermal adsorption and inhalation). In assessing the human health risk, the standard reference values provided by international organizations were used for the calculation of the Exp_{ing} humans, especially the adults, to heavy metals through the ingestion pathway.[1,14,22] Hazard quotient values for Cd and Pb in bivalves and gastropods molluscs were below 1. HQ<1 indicates no concern for adverse health effects or non-carcinogenic effects on human health.[14,21,1] Hence, the consumption of the molluscs presented in this study poses no risk to human health.

The result of this study is almost the same as the study of Losasso *et al*.[23] The heavy metal (Cd, Pb and Hg) exposure from shellfish consumption such as Manila clams (*Ruditapes philippinarum*) and grooved carpet shell (*Ruditapes decussatus*) in Veneto Region, North-eastern Italy, were examined. Pb has the highest concentration level in the shellfish studied. A food frequency consumption survey was also conducted for estimating the relative contribution of shellfish to the Cd weekly intake. The results of their study showed that the calculated THQ or total hazard quotient of Cd, Pb and Hg from 2007 to 2012 (THQ= 0.024, 0.020, 0.017, 0.016, 0.030 and 0.022) did not exceed the standard value 1.

However, in the study conducted by Denil, Fui and Ransangan[1] revealed that consumption of different bivalve species that are contaminated with heavy metals (Cu, As, Pb, Mn, Cd, Zn) was expected to bring health risk. These species include green mussel (*Perna viridis*), Asiatic hard clam (*Meretrix meretrix*), Pacific oyster (*Crasso strea gigas*) and marsh clam (*Polymesoda expansa*). This is based on the Total Hazard Index (THI) values calculated higher than the permissible limit 1.0 (THI = 1.60, 5.07, 1.86 and 4.40).

Chronic daily intake is the amount of chemical to which a person can be exposed each day for a long time without suffering harmful effects.[24] In this present study, CDI of Pb from the four marine molluscs was slightly higher compared to the CDI of Cd. It has to be noted that heavy metals or metalloids disturb cellular activities. It promotes oxidative stress due to the initiation of reactive oxygen species (ROS), causes damage to DNA, alters the normal function of the cell membrane and the absorption of nutrients and promoting malfunction of proteins. It also interferes with the proper functions of several organ systems.[1]

Potential carcinogenicity of metals is the primary concern nowadays. Through the continuous use of different metals in industries as well as in everyday living, the existence of problems regarding toxic metal pollution is expected. Exposure to these toxic metals affects an individual's health and the cause of acute and chronic toxicities to humans. Chronic exposure to heavy metal (Pb) affects the human nervous system, hematopoietic system and renal. Exposure to Pb also causes encephalopathy, peripheral neuropathy, central nervous disorders and anemia. Chronic exposure to Cd, on the other hand, affects renal, skeletal and pulmonary organs. Proteinuria, glucosuria, osteomalacia, aminoaciduria and emphysemia are also the harmful effects of Cd on humans.[25]

Carcinogenic risks (CR_{ing}) of heavy metals through marine mollusc consumption were evaluated to determine whether a certain chemical probably pose cancer risks or not, to humans. The CR_{ing} values for both Cd and Pb in this study were less than and within the permissible range, respectively. Hence, Cd and Pb in the marine mollusc tested may pose no potential carcinogenic risk to humans.

Physico-chemical characteristics of seawater

Diverse and unique aquatic organisms are found in marine waters. The existence of marine life forms is influenced by marine waters' most important components, which include temperature, pH, dissolved oxygen, salinity and nutrients.[26] Seawater temperature is one of the important factors to consider because this affects other parameters of seawater and as well as the organisms' survival.[5,26] The temperature obtained (30°C) as compared to the "Water Quality Guidelines and General Effluents Standards of 2016" by Department of Environment and Natural Resources (DENR) Administrative Order (DAO) No. 2016-08[27] was within the standard temperature range of 26-30°C for marine waters that are classified as protected water. This classification includes national or local marine parks, reserves and sanctuaries. With these, the temperature in Guang-guang is suitable for marine molluscs' survival.

A pH range of 6.5 to 9 is most appropriate for the survival of fish communities and other aquatic organisms.[5,26] Moreover, the measured dissolved oxygen was higher than the standard dissolved oxygen value of 6 mg/L. A higher dissolved oxygen level usually shows better water quality and is essential for marine organisms.[28] The dissolved oxygen level obtained shows that Guang-guang has a good marine water condition.

Furthermore, the TDS readings were still within the acceptable range of 30,000 to 40,000 ppm, according to the Water Quality Association.[29] In the marine ecosystem, total dissolved solids or TDS is also one of the factors that define the quality of water and its influence on marine organisms. Dissolved solids are any minerals, salts, metals, cations or anions dissolved in the water. An increased level of TDS indicates that water has a higher amount of chemicals, minerals, or salts.[30]

Antibacterial activities of marine mollusk

The three mollusks, *A. maculosa, A. puerpera and L. lambis*, showed weak inhibition against *S. aureus* and *E. coli*. On the other hand, *C. urceus* showed inhibition but only for *S. aureus*. The natural products of *C. urceus* must be investigated to know the secondary metabolites that might be responsible for the exhibited antibacterial activity. Moreover, different extracting solvents can be considered to maximize the extraction of the different secondary metabolites that have an antibacterial factor. A study conducted on the egg strings and ink of a gastropod, *Dolabella auricularia* from Pujada Bay, gives promising results on its antioxidant and antibacterial activities using solvents with different polarities. These are greatly attributed to the presence of secondary metabolites in the tested species and its synergistic effects.[31-33]

CONCLUSION

The Cd and Pb concentrations in the four mollusks species, namely; *A. maculosa, A. puerpera, C. urceus* and *L. lambis* collected from Guang-guang, Pujada bay, Davao Oriental were below the standard limit of 0.5 µg/g set by FAO No.210 (2001) and other international agencies. The health risk indices were all below the acceptable level set by USEPA. Hence, Cd and Pb posed no potential non-carcinogenic and carcinogenic risks to human health and are safe for consumption. The seawater quality was good, based on the determined physico-chemical characteristics of the water. Lastly, *C. urceus* showed an antibacterial factor. Further studies on its natural products are highly recommended.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

RQ: Risk Quotient; **HQ:** Hazard Quotient; **CDI:** Chronic Daily Intake; **CR:** Carcinogenic Risk; **FAO:** Fisheries Administrative Order.

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