Bioaccumulation potential of marine bivalve seashells on heavy metals along the intertidal zone of selected coastal barangays in malita davao occidental, Philippines

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ABSTRACT

This project has two components namely: Project 1 on assessment of marine bivalve seashells and Project 2 on bioaccumulation potential of heavy metals. It was conducted to determine species density, abundance and sizes of marine bivalve seashells, substrate type and the concentration of heavy metals from the collected tissue samples. Transect Line Quadrat Method was used to assess marine bivalve seashells while the Atomic Absorption Spectrophotometer (AAS) was used to quantify the concentration of heavy metals. There were 13 species of bivalve seashells identified with *Anadara satawi* which exhibited highest the density and relative abundance, whereas, *Gafrarium tumidum* obtained the highest mean for length and width. However, Fine sands substrate exhibited the highest percentage value and was considered as major substrate type in the project areas. In terms of the bioaccumulation potential of marine bivalve seashells on heavy metals, *Anadara sp.* were observed to have concentration of Zinc, while concentration of Copper was high in *Grafrarium sp.* However, Lead and Mercury were observed with lowest concentrations in all project sites.

Keywords: atomic absorption spectrophotometer, bioaccumulation, heavy metals, marine bivalves seashells, transect line quadrat method.

1 INTRODUCTION

Marine bivalve seashells are the second of the most diverse and dominant groups of marine invertebrates from the Phylum Mollusca, the term “Bivalve” is used for the reason that the external shell is composed of two valves to make it as one shell by Salamanca and Pajaro (1996), according to Tucker and Dance (1982) they are commonly called clams, cockles, mussels and oysters which their shells are made up of calcium carbonate.

Marine bivalve seashells are known as bio-indicator organisms to assess water condition (Wilson, 2008), they have the potential to accumulate heavy metals that are initiate in seawater (Nybakken, 1982). However, heavy metals are parameters in monitoring human activities and their potential effects on marine ecosystem (Greenpeace Southeast Asia, 2007).

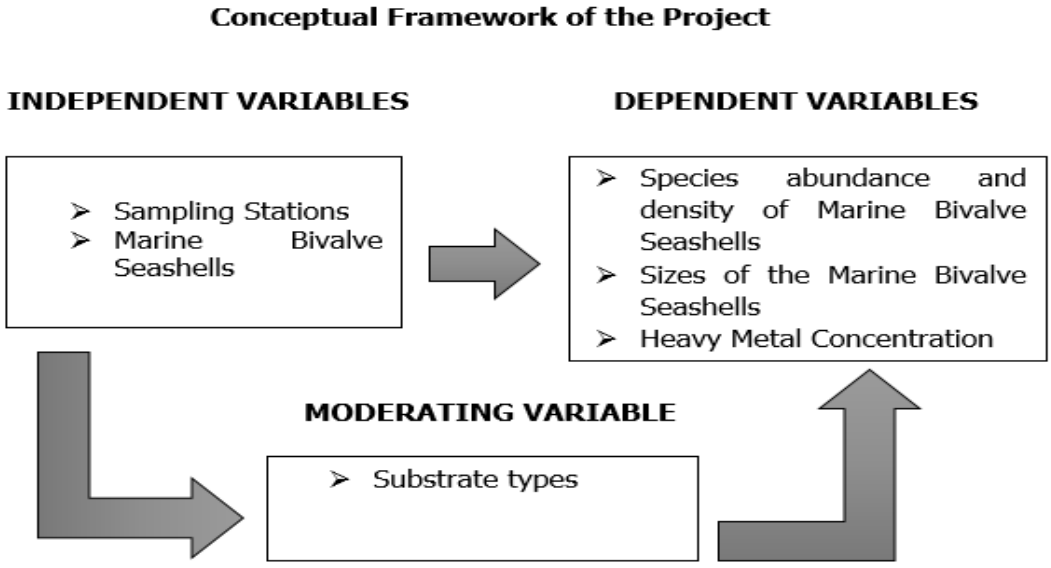
It is interesting to note that assessment of marine bivalve seashells are not just indispensable for biological and commercial prominence but also for scientific research purposes. With this, an information drive on the project findings were disseminated to the community so that they have initial information to preserve marine resources against exploitation and toxic pollution.

2 OBJECTIVES

The focus of this project was to assess the marine bivalve seashells and bioaccumulation potential of marine bivalve seashells on heavy metals along the intertidal zone of the selected coastal barangays in Malita, Davao Occidental, Philippines. Specifically, the project aims to:

1. Identify the species of marine bivalve seashells and calculate density and abundance along the intertidal zone of the selected barangays in Malita, Davao Occidental;
2. Determine the sizes of marine bivalve seashells found in the project area;
3. Characterization of the substrate type in the project area;
4. Determine the concentration of heavy metals such as Copper (Cu), Lead (Pb), Mercury (Hg) and Zinc (Zn) in the tissue samples of marine bivalve seashells collected from the project areas.

Figure 1. Schematic diagram showing the relationship between the independent and dependent as well as moderating variables of the project.



The sampling stations and marine bivalve seashells were considered as the dependent variables because marine bivalve seashells are organism that manifestation to verify the water condition of the project areas. While, the species abundance and density, sizes of the marine bivalve seashells and heavy metals concentration such as Copper (Cu), Lead (Pb), Mercury (Hg) and Zinc (Zn) was considered as the independent variable, they are directly affected of many attributes to the seawater such as pollution towards anthropogenic activities. However, substrate types were considered as moderating variable of the projects.

3 MATERIALS AND METHODS

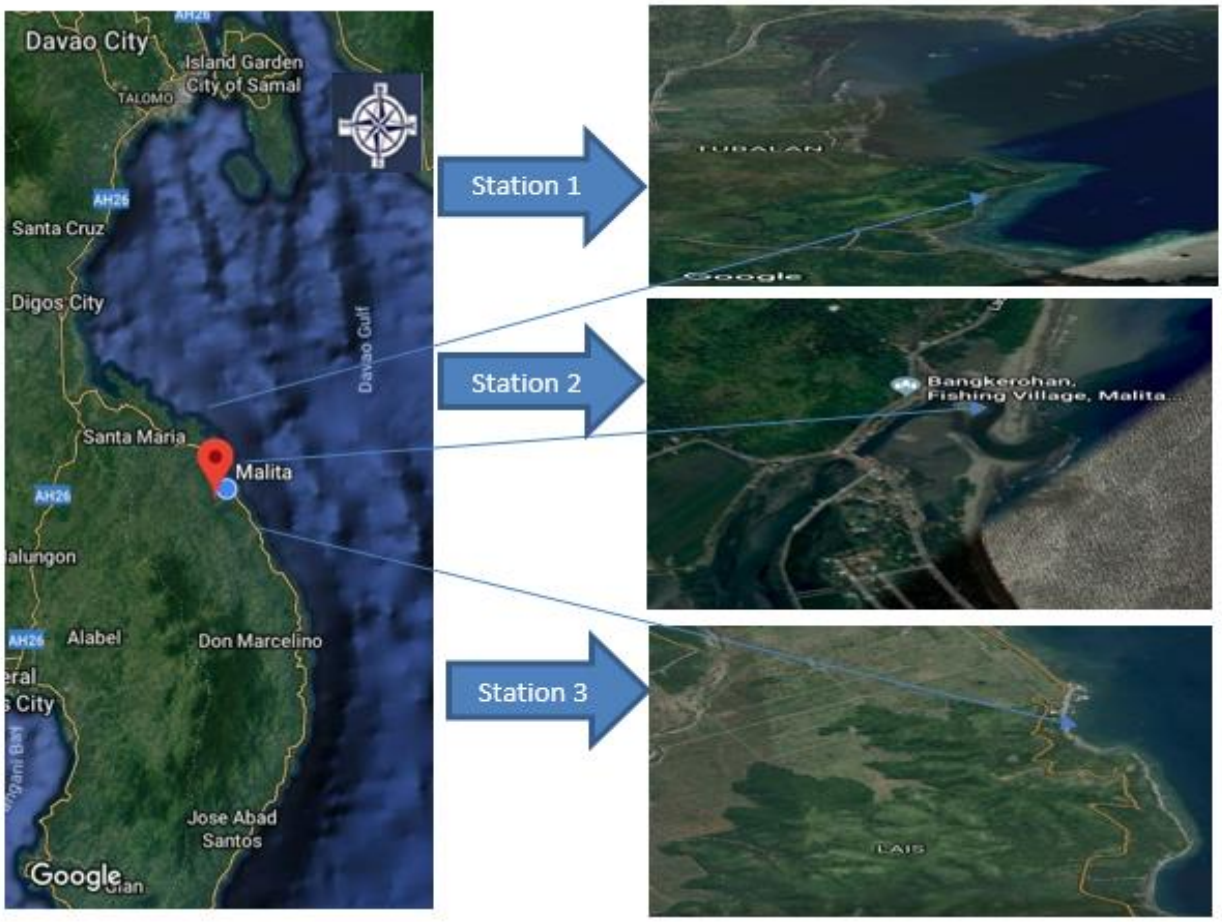
Research Locale:

The project was conducted along the intertidal zone of selected coastal barangays in Malita, Davao Occidental, Philippines where mangrove forest, seagrass beds and coral reefs are diverse in the project area, due to its economic development many coastal barangays in the project areas had been converted to industrial and residential property.

Table 1. Location of the selected project area.

Station Area	Barangay	Coordinates
1	Tubalan	North 06°29.370', East 125°35.045
2	Fishing Village	North 06°26.332', East 125°35.014
3	Lais	North 06°19.465', East 125°39.211

Fig. 2. Map of the project area.



4 PROJECT 1. ASSESSMENT OF MARINE BIVALVE SEASHELLS

4.1 RESEARCH DESIGN AND INSTRUMENTS

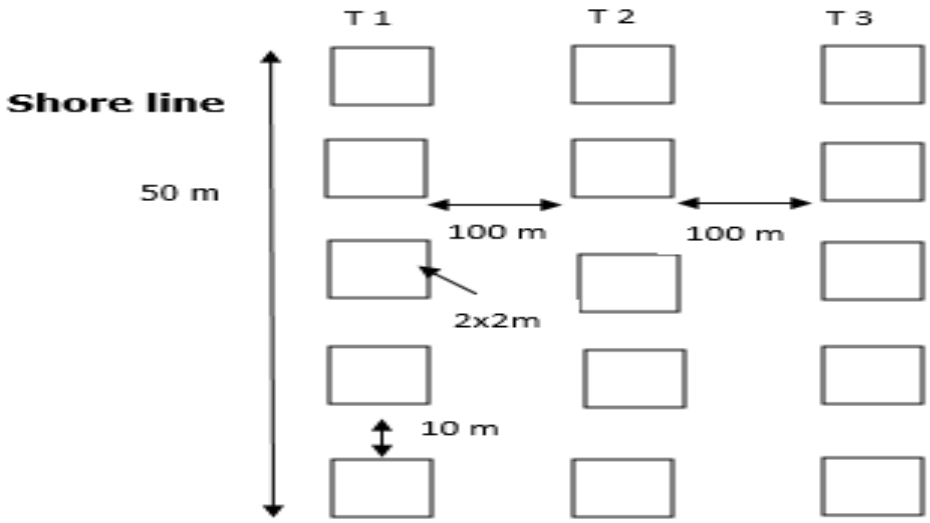
For project 1 on assessment of marine bivalve seashells, data on species density and abundance of marine bivalve seashells along the intertidal zone in the project areas were gathered through Transect Line Quadrat Method and one shot sampling technique, and calculated using the formula of (Odum, 1971), while data on substrate types in the project areas were gathered using different sieve mesh and calculated using the formula of (Kaye,1985), gram size classification was identified using the identification chart by (Whiting *et al.,* 2010), data on actual sizes of bivalve seashells were gathered using the vernier caliper and identification of marine bivalve seashells was used by the reference book of Laureta (2008) and Oliver (2004).

4.2 ESTABLISHMENT OF THE SAMPLING AREA

Preliminary survey was done in the project area to determine the different species of marine bivalve seashells while other species were considered as subject for this projects. There are three stations

were established in the project area namely: Station 1 (Barangay Tubalan), Station 2 (Barangay Fishing Village) and Station 3 (Barangay Lais).

Fig. 3. Field sampling design of the project.



5 PROJECT 2. BIOACCUMULATION POTENTIAL ON HEAVY METALS

5.1 EXPERIMENTAL DESIGN

Complete Replicate Design with Complete Replication was used in the project to determine the comparison of samples and significant difference of the project.

Table 2. Experimental Treatment of the Project

STUDY AREA	SAMPLES/ REPLICATE	CONCENTRATION/TREATMENTS				
		[0.2 ppm]	[0.5 ppm]	[1 ppm]	[2.5 ppm]	Blanks
1 BARANGAY TUBALAN	Sample 1 (Cu) (Pb) (Hg) (Zn)					
	Sample 2 (Cu) (Pb) (Hg) (Zn)					
	Sample 3 (Cu) (Pb) (Hg) (Zn)					

2 BARANGAY FISHING VILLAGE	Sample 1 (Cu) (Pb) (Hg) (Zn) Sample 2 (Cu) (Pb) (Hg) (Zn) Sample 3 (Cu) (Pb) (Hg) (Zn)
3 BARANGAY LAIS	Sample 1 (Cu) (Pb) (Hg) (Zn) Sample 2 (Cu) (Pb) (Hg) (Zn) Sample 3 (Cu) (Pb) (Hg) (Zn)

5.2 SAMPLE PREPARATION AND TISSUE EXTRACTION

The 5 grams of tissue samples were digested and soaked of 10 mL concentrated nitric acid for 24 hours using open glass container for the pre-treatment of the samples. Treated and digested samples were filtered using whatman 0.42µm filter paper into a 50 mL volumetric flask, diluted samples were stored in polyethylene (PET) bottles and the analysis were done using Atomic Absorption Spectrophotometer (Solidum *et al.*, 2013).

5.3 PREPARATION OF REAGENTS

Concentration levels of 0.2 ppm, 0.5 ppm, 1 ppm, 2.5 ppm and blank of standard solution in every heavy metals were prepared to calibrate Atomic Absorption Spectrophotometer to quantify the concentration of heavy metals in tissue samples of marine bivalve seashells collected from the project area. The project were used different wavelength such as Copper (Cu) have a wavelength of 324.8 nm, Lead (Pb) had 405.8 nm, while Mercury (Hg) with 253.7 nm and Zinc have a wavelength of 636.2 nm to determine the concentration of specific heavy metals.

5.4 STATISTICAL ANALYSIS

Data were calculated using Analysis of Variance and Kruskal-Wallis H test to determine significant differences of the project. Tukey's Honesty Test by (Daniel, 1995) for significant different when observed in the project. However, the analysis were calculated and checked using SPSS 17.0.

6 RESULTS AND DISCUSSIONS

6.1 MARINE BIVALVE SEASHELLS IDENTIFIED

There were 13 species of marine bivalve seashells identified in the project area. (Table 3). Among the species both *Circe scripta* and *Gari togata* were observed in the three study area, possible factors are due to common substrate type of fine muddy and sandy sand in the sampling area. Project of Laureta (2008) confirmed both *Circe scripta* and *Gari togata* are distributed of the three stations in muddy sediments and fine sand which this type of substrate caused in this project. However, project of Mcdonald *et al.,* (*2015*) human disturbances along coastal areas were declined the habitat that could affects the distribution of species density of the marine bivalve seashells and complicate the ecosystem, while, a project conducted by Boera (2001) on the impact of human activities confirms that the anthropogenic events on the marine bivalve seashells were decreased on its density, abundance and diversity.

Table 3. Occurrence of marine bivalve seashells in the project area.

MARINE BIVALVE SEASHELLS SPECIES	BARANGAY TUBALAN	BARANGAY FISHING VILLAGE	BARANGAY LAIS
Anodontia eduntula	-	/	/
Anadara satawi,	/	-	-
Circe scripta	/	/	/
Donax cuneatus	-	-	/
Gafrarium tumidum	/	/	-
Gari togata	/	/	/
Laterna truncata	-	/	-
Lioconcha castrensis	-	-	/
Lioconcha fastigata	-	-	/
Lioconcha ornata	-	-	/
Paphia gallus	-	-	/
Tellina straurella	-	-	/
Venerupis philippinarium	-	-	/

Legend: - = denote absents
/ = denote presents

6.2 DENSITY AND ABUNDANCE OF MARINE BIVALVE SEASHELLS

Data on density and relative abundance are presented in Table 4. Results showed that in Station 1 the *Anadara satawi* obtained the highest density of 391 m^2 and relative abundance of 69.46%, Station 2 the *Gafrarium tumidum* exhibited the highest density of 244 m^2 and relative abundance of 56.46 %, while in Station 3 the *Lioconcha fastigata* attained the highest density of 2.85 m^2 and relative abundance of 31.15%, respectively.

Table 4. Species density (individual/4 m^2) and relative abundance (%) of marine bivalve seashells in the project area.

STATION		SPECIES	DENSITY	ABUNDANCE
1	BARANGAY TUBALAN	*Anadara satawi*	391	69.46
		Circe scripta	36	6.39
		Gafrarium tumidum	121	21.49
		Gari togata	15	2.66
2	BARANGAY FISHING VILLAGE	*Anodontia eduntula*	7	1.59
		Circe scripta	88	20
		Gafrarium tumidum	244	56.46
		Gari togata	77	17.5
		Laterna truncate	24	4.45
3	BARANGAY LAIS	*Circe scripta*	87	9.50
		Donax cuneatus	26	2.84
		Gari togata	260	28.42
		Lioconcha castrensis	89	9.73
		Lioconcha fastigata	285	31.15
		Lioconcha ornata	68	7.43
		Paphia gallus	31	3.39
		Tellina straurella	41	4.48
		Venerupis philippinarium	28	3.06

Among the three stations Brgy. Lais exhibited the lowest density and abundance but represents the highest composition of marine bivalve species identified in the project area. A project of Laureta (2008) throughout Panay explained that numerous species of marine bivalves were found thriving in shallow waters mostly where coral reef and mangrove forest are situated. Similar with that of Brgy. Tubalan and Brgy. Fishing Village where these mentioned marine ecosystems are existent. Furthermore, a more complex environment conditions observed in Iloilo with the presence of longer coastline and variable bottom sediments and large fringing reefs could be contributory factors of more species of marine bivalve identified which observations are comparable with that in Brgy. Lais.

There is no significant difference on the species density and abundance of marine bivalve seashells along the intertidal zone of the selected barangays in Malita, Davao Occidental, Philippines. This could be attributed to the more or less similar conditions of the three sampling stations in terms of substrate types, temperature and salinity. This is to support the statement of Waddy and Aiken (1993) stated marine bivalve seashells are abundant along intertidal zone occurring in different habitat such as coral reefs, rocky coast, sandy beaches, seagrass beds and even in depth seawater.

6.3 SIZES OF MARINE BIVALVE SEASHELLS

In terms of length and width of the marine bivalve seashells, the results showed that *Anandara sp.* obtained the highest value of 1.1 cm in length and 1.5 cm in width at station 1, while the *Gafrarium sp.* exhibited mean value of 1.7 cm in length and 2.6 cm in width at station 2, likewise, *Gari sp.* obtained utmost value of 0.8 cm in length and of 1.1 cm in width at station 3, respectively (Table 5).

Table 5. Mean of length (cm) and width (cm) of the bivalve seashells in the project area.

	STATION	SPECIES	MEAN OF LENGTH	MEAN OF WIDTH
1	BARANGAY TUBALAN	*Anadara satawi*	1.1	1.5
		Circe scripta	0.5	0.8
		Gafrarium tumidum	0.5	0.8
		Gari togata	0.3	0.6
2	BARANGAY FISHING VILLAGE	*Anodontia eduntula*	0.2	0.3
		Circe scripta	1.3	2.1
		Gafrarium tumidum	1.7	2.6
		Gari togata	0.7	2.4
		Laterna truncata	0.7	1
3	BARANGAY LAIS	*Circe scripta*	0.5	0.8
		Donax cuneatus	0.3	0.6
		Gari togata	0.8	1.1
		Lioconcha castrensis	0.3	0.6
		Lioconcha fastigata	0.4	0.6
		Lioconcha ornata	0.3	0.5
		Paphia gallus	0.1	0.2
		Tellina straurella	0.2	0.3
		Venerupis philippinarium	0.1	0.2

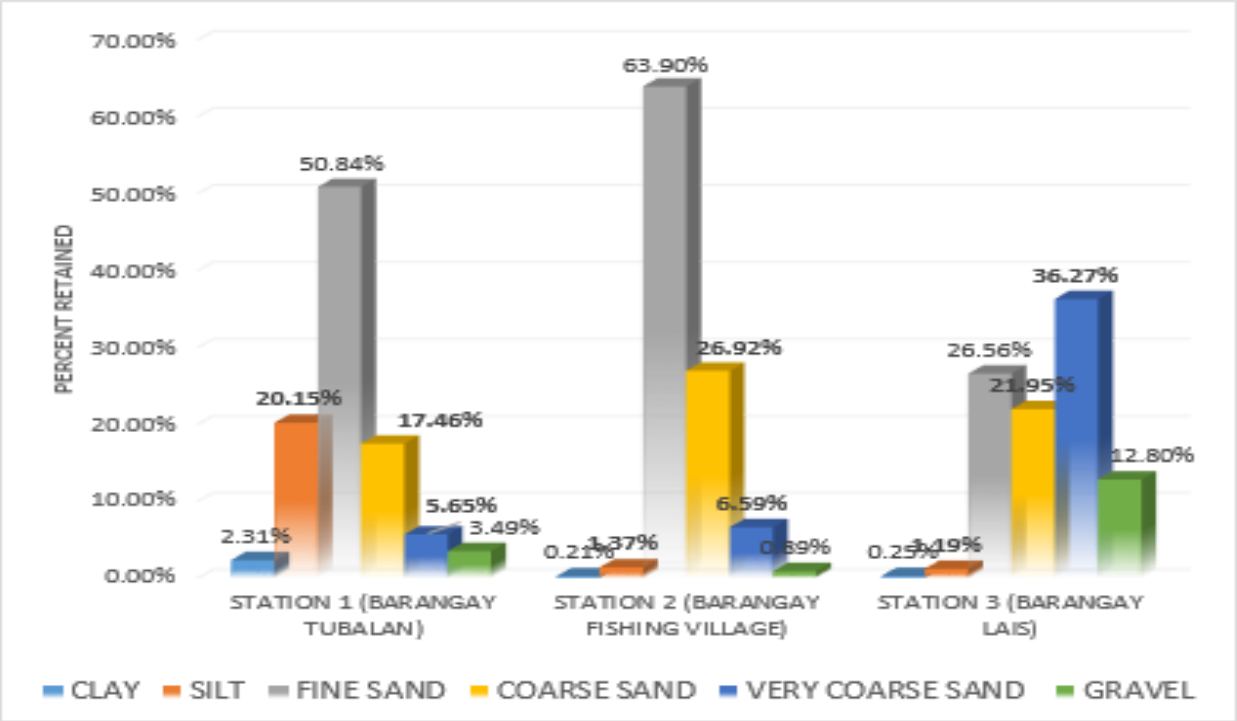
Comparable project of Laureta (2008) reported the size of marine bivalve seashells like *Laterna sp.* had 5.5 cm, *Anodontia sp.* had 6 cm, *Anadara sp*. had 6 cm, *Circe sp.* 4.6 cm, *Gari sp.* had 7.2 cm and *Gafrarium sp.* had 4. cm, while *Lioconcha sp.*, Paphia and Tellina had 3.5 to 4 cm, Donax had 2.5 cm, *Venerupis sp.* had 3.5 cm. Obviously, the presented data from Table 5 is lower than the reported data of Laureta (2008). The reduced size of some marine bivalves seashells observed in this project as composed to the findings of Laureta (2008) particularly *Anadara sp., Anodontia sp., Circe sp., Donax sp. Gafrarium sp., Gari sp., Laterna sp., Liochoncha sp., Paphia sp., Tellina sp.* and *Venerupis sp.* is an indicator of over exploitation of the commodity. The findings were confirmed by the project of Salamanca and Pajaro (1996) in Palawan observed that marine bivalve seashell sizes have been decreased and smaller over the years, this reduction in quantity and sizes attributed to increase consumption by growing human population, recent shift by marine bivalve seashells gleaners to fishing activity is an indicator of overexploitation of marine bivalve seashells resources that not been regulated.

6.4 SUBSTRATE TYPE IN THE PROJECT AREA

Station 1 in Barangay Tubalan found out the percent retained of fine sands substrate of 50.84%, while Station 2 in Barangay Fishing Village fine sands substrate of 63.90 % and Station 3 in Barangay Lais exposed percent coarse sands substrate (36.27 %), respectively (Figure 4).

Fine sand and coarse sand substrate type could be favorable habitat of the marine bivalve seashells at the three sampling station in the project area, particularly, *Anadara sp., Anodontia sp., Circe sp., Donax sp., Gafrarium sp., Gari sp., Laternula sp., Lioconcha sp., Paphia sp., Tellina sp.* and *Venerupis sp.*

Fig 4. Substrate type of the project area.



This findings supported the project of Tyler (2007) stated that marine bivalve seashells are buried in different substrate type such as sand, muddy sand, muddy gravel. A similar project of Poutiers (1998) confirmed that *Anadara sp.* and other cockles found in large numbers in mudflats while comparable study of Laureta (2008) ratify that marine bivalve seashells like *Laterna sp., Gari sp., Circe sp., Anodontia sp.* are habitat and distributed in muddy sediments, fine sand types of substrate, muddy estuaries, mangrove areas and brackishwater ponds, furthermore, marine bivalve seashells like *Donax sp., Lioconcha sp., Ruditapes sp., Gafrarium sp.* habitat and distributed in sandy bottom of shallow water, sand flats and coral areas, beach slope and sand-mud estuaries.

6.5 BIOACCUMULATION POTENTIAL ON HEAVY METALS

Data on the concentration of heavy metals in the tissue samples in Table 6. The results showed that Zinc (Zn) had highest concentration of 12.0186 ppm in Barangay Tubalan , while Copper (Cu) had highest concentration of 1.6485 ppm in Barangay Fishing and Zinc (Zn) had highest concentration of 11.257 ppm in Barangay Lais. While, Lead (Pb) and Mercury (Hg) having the lowest concentration. These values are still lower that minimum permissible limits such as Copper (Cu) have only acceptable with 2

mg/kg, Lead (Pb) have 0.3 mg/kg, Mercury (Hg) have 0.01 mg/kg and Zinc (Zn) have 10 mg/kg (Codex Alimentarius, 2015). Detection of these heavy metals is alarming or an indicator that there are contributory factors that influencing the accumulation of the heavy metals in the project area. The contributory factors could be probably the coal-fired power plant, banana plantation, aquaculture and mericulture production and urbanization. The effluent discharges, non-point sources, agricultural runoff from rivers and running freshwaters were observed in the project area during field sampling, with this observation a project of Greenpeace Southeast Asia (2007) confirmed that heavy metals from industrial sources, agriculture and urbanization were found to contribute the concentration of heavy metals on seawater pollution in Mindanao.

Table 6. Concentration of heavy metals in the tissue samples of marine bivalve seashells collected from project areas.

STUDY AREA	TISSUE SAMPLES		HEAVY METALS/ CONCENTRATION (ppm)				
			COPPER (Cu)	LEAD (Pb)	MERCURY (Hg)	ZINC (Zn)	MEAN
1 BARANGAY TUBALAN	*Anadara sp.*	1	1.1721	0.91111	0.0029	10.5170	3.1508
		2	1.9015	0.7709	0.0056	12.0186	3.6742
		3	1.2516	0.8293	0.0036	0.4509	0.6339
2 BARANGAY FISHING VILLAGE	*Gafrarium sp.*	1	1.6485	0.8996	0.0041	0.0639	0.654
		2	1.6665	0.9139	0.0047	0.9900	0.8938
		3	1.4680	1.1939	0.0045	1.1134	0.945
3 BARANGAY LAIS	*Lioconcha sp.*	1	1.2035	1.0634	0.0043	3.4571	1.4321
		2	1.8085	1.1268	0.0043	11.2403	3.545
		3	1.7603	1.0647	0.0043	3.9540	1.6958

These possible factors could contribute the findings on concentration among heavy metals found in tissue samples of marine bivalve seashells of the sampling station areas. On the other hand, a similar project of Louma *et al.*, (1984) other factors and influence on concentration of heavy metals in terms of sampling seasons, chemistry of surrounding water and sediments by Graney *et al.*, (1984).

The findings of the project with high concentration of Copper (Cu) and Zinc (Zn) in the project area was supported to the reports of Environmental Management Bureau (2006) found out background concentration of heavy metals in Meycauayan River with shows excess minimum criteria of Copper (Cu) and Zinc (Zn) in creeks and rivers at sampling station, similarly, this reports found out with the same effluent discharges and contributors like running waters in project area. Moreover, a project of Environmental Management Bureau (2006) reported in Marilao River showed excess relative existing standard in Lead (Pb) of 190 ppm due to manifestation of tanneries and electroplating factories and

continuous monitoring of Mercury (Hg) in small scale mining area in some parts in Eastern Mindanao reported high concentration of Mercury (Hg) levels in Naboc River.

Comparable of Wilson (2008) stated that on biodynamic theory predicts seasonal differences on concentration of heavy metals based on the metallic activity and source of food such as seasonal algal blooms, where the sampling period of the project was held during dry seasons which could be factor to determine the concentration of heavy metals in the project site. Tissue samples of marine bivalve seashells like *Anadara sp., Gafrarium sp.* and *Lioconcha sp.* are worthy specimen to quantify the concentration of heavy metals such as Copper (Cu), Lead (Pb), Mercury (Hg) and Zinc (Zn) through bioaccumulation processes and activity of substances intake by these marine bivalve seashells species. A project of Shirneshan and Bakhtiar (2012) on accumulation of heavy metals confirmed that the tissue sample of oyster accumulated of heavy metals like Copper (Cu), Lead (Pb), Mercury (Hg) and Zinc (Zn) confirmed that this high content in the tissue samples of marine bivalve seashells may be due to the presence of cysteine.

7 SUMMARY

Thirteen (13) species of marine bivalve seashells were identified in the project area namely: *Anodontia eduntula, Anadara satawi, Circe scripta, Donax cuneatus, Gafrarium tumidum, Gari togata, Laterna truncata, Lioconcha castrensis, Lioconcha fastigata, Lioconcha ornata, Paphia gallus, Tellina straurella, Venerupis philippinarium.*

Among the marine bivalve seashells, *Anadara satawi* obtained the highest density of 391 m^2 and relative abundance of 69.46% in station 1. There is no significant difference on the species density and abundance of marine bivalve seashells along the intertidal zone of the selected barangays in Malita, Davao Occidental, Philippines.

Among the marine bivalve seashells, *Gafrarium tumidum* obtained highest mean value of 1.7 cm in length and 2.6 cm in width in station 2. No significant difference on the sizes of marine bivalve seashells found in the project areas.

Among percent retained, fine sands substrate obtained highest value of 63.90% in station 2. There is no significant difference on the substrate type in the project areas.

Among heavy metals, Zinc (Zn) had the highest concentration of 12. 0186 ppm in station 1. There is no significant difference in concentration of the heavy metals such as Copper (Cu), Lead (Pb), Mercury (HG) and Zinc (Zn) in the marine bivalve seashell samples collected from the project areas.

8 CONCLUSIONS

The following conclusions were derived based on the findings of the project:

1. There are thirteen species of marine bivalve seashells found in the three station along the intertidal zone coastal barangays in Malita, Davao Occidental, Philippines.

2. *Anadara satawi* obtained the highest density of 97.75 m^2 and highest relative abundance of 69.46%, while *Anodontia edentula* has exhibited lowest density .of 1.75 m^2 and lowest relative abundance of 1.59%.

3. *Grafrarium tumidum* has the highest mean value of 1.7 cm in length and 2.6 cm in width while *Paphia sp.* and *Venerupis* has lowest mean value of 0.1 cm in length and 0.2 cm.

4. Fine sand substrate has the highest percent retained of 63% at station 2 while clay substrate has the lowest percent retained of 0.21% at station in the project area.

5. Zinc (Zn) accumulation to *Anadara sp.* is reported highest concentration of 12.0186 ppm at station area 1 in Barangay Tubalan, followed by Zinc (Zn) to *Lioconcha sp.* has described highest concentration of 11. 2403 at station area 3 in Barangay Lais, and Copper (Cu) accumulation to *Grafrarium sp.*is obtained highest concentration of 1.6685, while Lead (Pb) 0.7709 ppm and Mercury (Hg) 0.0043 ppm has found lowest concentration from the collected marine bivalve tissues in the project area.

RECOMMENDATIONS

Based on the result of the project the following recommendation are suggested:

1. Assess the resource status of marine bivalve seashells along the intertidal zone in Davao Occidental, Philippines.

2. Further study on bioaccumulation potential of marine bivalve seashells in absorbing other heavy metals such as Nickel, Cadmium and Arsenic during wet and dry seasons along the intertidal zone in Malita, Davao Occidental, Philippines.

3. Conduct a project on concentration of heavy metals collected shells and substrates samples at coastal barangays in Davao Occidental, Philippines.

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