Assessment of Heavy Metals in Philippine Green Mussels *Perna viridis* and Level of Coliform on Manila Bay Adjacent to the Coastline of Sipac Almacen, Navotas Philippines

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***Abstract*— *There have been no published data reports up to date regarding the heavy metal concentration on both the green mussels Perna viridis and total coliform level from Manila bay adjacent to Sipac Almacen, Navotas Philippines. Hence, this study aimed to provide a recent status on the concentration of heavy metals (Cd, Cr, Pb, and Hg) in the muscular tissues of P. viridis and coliform level from Manila Bay, Philippines. Specimen samples were collected on February 22, 2019, almost 1000 m away from the coastline, immediately brought to the laboratory, morphologically identified, dissected for muscles, and subjected to heavy metal and coliform testing. Tissue samples of P. viridis and sediment samples from Manila bay were subjected to Flame-AAS (atomic absorption spectrophotometry) method for detecting and quantifying heavy metals such as total cadmium (Cd), total chromium (Cr), and total lead (Pb) while the analysis of total mercury (Hg) were done using the Cold Vapor-AAS method. With reference to a previous study, the sediment and P. viridis from this study obtained a lower total Cd, Cr, and total Pb while total Hg concentration is below the resulting limit. The water sample was subjected to multiple fermentation technique to identify the coliform level which shows a high-level result of 1.6 X 103 that is far from the 3000 value set by DAO 2016-08 for SB water body category. The physicochemical analyses on the bay show no value of ecological concern. P. viridis in Manila Bay did not exhibit any serious deformities*

***Keywords*— Manila Bay, heavy metal, Portunus pelagicus, AAS, pollution.**

I. INTRODUCTION

Navotas is popularly known as the fishing capital of the Philippines. It has a total land area of 10.77 km^2. It is considered as a coastal town in the northwest part of Metro Manila. It is a tapering strip of land with an aggregated shoreline of approximately 4.5 km. In the north, Navotas has a common boundary with the town of Obando, Bulacan, along Sukol Creek which separates it from Balt. Along the eastern border runs the Binuangan River, the Daang Cawayan River, the Dampalit River, the Batasan River, the Navotas River, the Bangculasi Channel, the Malabon Channel and the Estero de Maypajo (PSGC 2018).

Navotas river is the major channel in the city of Navotas. It Intersects with Tullahan river that drains water from Navotas, Caloocan, and Malabon and dumps water directly to Manila bay adjacent to the coastline of Sipac Almacen Navotas, Philippines.

Navotas city is a highly urbanized city in Metro Manila (Census of Population (2015), and has a population of 249,463 people (Census of Population (2015). Informal settlers are generally those who occupy lands without the consent of the property owner (Reyes et al 2012, 15).

Informal settlers living in the coastline of Sipac Almacen, Navotas facing Manila bay area are families of the fishermen, fish vendors in the fish port, fish porters and skippers (bangkero). In Manila, they are commonly called as urban poor. However, not all of them are poor because they have an occupation to pay bills. Poor sanitary discipline was observed in the area, no appropriate toilets that dispose of human wastes, many biodegradable and non-biodegradable wastes found uncontrollably floating on the water near the residential area. Since Sipac Almacen is a residential area that is across the breakwater when it is high tide water mixes with the Navotas river which presumably the cause of the high level of coliform. Approximately, 400 m away from the coastal area of Sipac Almacen, Navotas facing Manila Bay, a number of docked Cargo ships and Fishing vessels were commonly found along the Manila Bay. Some of the cargo ships and fishing vessels were drydocked for repair. Presumably, the crews of the shipping vessels and cargo ships dumped liquid wastes in the Manila Bay. Thus, a high possibility for heavy metal contamination.

According to Table 2 DAO 2016-08 of Water Quality Guidelines and Effluent Standards of 2016 of Water Body Classification and Usage of Marine Waters, Manila bay falls under the classification of SB, wherein it states that this water body is suitable for commercial propagation of shellfish and spawning areas of milkfish and similar species, it serves as ecotourism and recreational activities spot.

Perna viridis is commonly found at the tropical and subtropical regions and reproduce rapidly even at extreme conditions like polluted harbours or bays just like on the results of the study of Rajagopal et al. (1997, 1998b) where very high densities of *P. viridis* have been reported from polluted harbours and submarine pipelines of coastal power stations. With this special reproduction behaviour of the *Perna viridis,* a lot of local fishermen invest on culturing it on the coastal area of Sipac Almacen, Navotas Philippines even if there are a lot of cargo ships, vessels, and informal settlers.

Culturing mussels at the intertidal zone of Sipac Almacen, Navotas is a common scenery. *Perna viridis* is not only part of their meal plan likewise it is a source of income for local fishermen. Due to increasing prices of the basic commodities at the Philippines, people look for food source which has a high nutritive value but on a cheaper price like *Perna viridis. Perna viridis* is rich in amino acids, vitamins A, B_1, B_2, B_3, B_6, B_{12}, and C. It is likewise rich on both macro and micro mineral contents. The macro mineral calcium, potassium, sodium and iodine were found to be high. Magnesium and iron were significantly detected in meager level. Trace metals like zinc and copper were in trace level (Saritha 2015).

The Aim of the Study

It is better to understand that metal test is very crucial in interpreting the quality of water, that is why the very aim of this study is to assess the quality of water using Perna viridis as a biological indicator and sediment samples as recipient of different depository materials from numerous anthropogenic sources on Manila Bay for metal test and to determine the level of coliform in the coastline of Sipac Almacen facing Manila Bay area.

Significance of the Study

This research is significant to the locals of Navotas, Caloocan, and Malabon to ensure the quality of water on Manila Bay performs its top ecosystem service that is a habitat for various marine life organisms, for example, Perna Veridis as a staple food for the people of Navotas, Caloocan, and Malabon. As to date, this is the only research study done in Manila Bay Adjacent to Sipac Almacen Navotas, Philippines.

II. MATERIALS AND METHODS

On February 5, 2019, an initial site analysis was conducted in Manila Bay Adjacent to Sipac Almacen Navotas Philippines, to determine if the water and the biological indicator Philippine Green Mussels Perna viridis is possibly contaminated with coliform and heavy metals.On February 22, 2019, 10:00 AM sample collection was conducted in Manila Bay Adjacent to the coastal area Sipac Almacen, Navotas Philippines approximately 14'34''15.20°N 120'56''31.85° E. Samples were collected, stored in a cooler, and immediately brought to the laboratory for processing.

Specimen samples were morphologically identified through www.sealifebase.org (Poutiers 1998). Samples for analysis were dissected, and muscular tissues were obtained for heavy metal testing. Tissue samples were subjected to Flame AAS (atomic absorption spectrophotometry) method for detecting and quantifying heavy metals such as total cadmium (Cd), total chromium (Cr), and total lead (Pb) following the standard procedures from AOAC International 19th ed. 2012. The analysis of total mercury (Hg) was done using the Cold Vapor-AAS method in reference to AOAC International 19th ed. 2012. All heavy metal analysis was performed at the F.A.S.T Laboratories Cubao, Quezon City, Philippines.

Sediments sample were collected and submitted immediately to the Laboratory for Metal test. Cold vapor AAS was used to test the presence of Mercury. Flame AAS was used to test the presence of the metals Cadmium (Cd) Chromium (Cr) and Lead (Pb) in reference to AOAC International 19th ed. 2012. 4500- O C. Azide Modification was used to determine the dissolved oxygen, argentometric for examining the salinity, 4500- H+ B electrometry/ 2550 B Laboratory and field was used to determine the Ph/ Temperature, 2120 B visual comparison for examining the color and 2130 B.

Nephelometric was used to determine the turbidity of the water sample. All these tests and procedures in reference to APHA AWWA and WEF 2012/2017 Standard Method for the Examination of Water and Wastewater 22nd and 23rd Edition. Multiple Tube Fermentation Technique (MPN/100ml) was used to determine the coliform level of the water sample in reference to APHA AWWA and WEF 2012/2017 Standard Method for the Examination of Water and Wastewater 22nd and 23rd Edition.

Description of the Sampling area

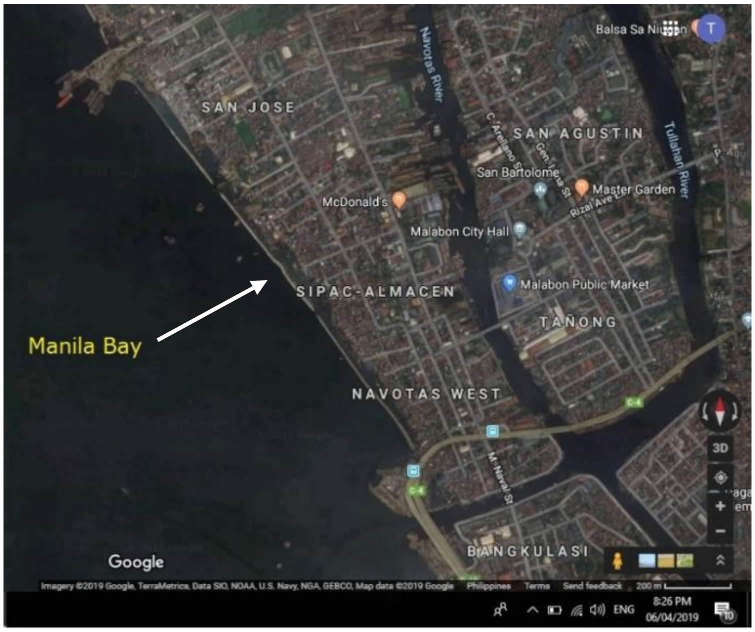

Fig.1: Geographic satellite view of the Manila bay Adjacent to Sipac Almacen, Navotas Philippines. Google Earth 2019.

On figure 1 shows the geographic satellite view of Manila Bay adjacent to the residential area of Sipac Almacen Navotas Philippines is bounded with two tributaries namely Navotas river and Tullahan river. With the coordinates, approximately 14'34''15.20°N 120'56''31.85° E. Number of huge docked ships for repair is commonly observed in the area. Fishing vessels are in abundance in line with business activities at the coastline area of Sipac Almacen Navotas. Informal settlers are seen at the very edge of the coastline of Sipac Almacen Navotas facing Manila Bay.

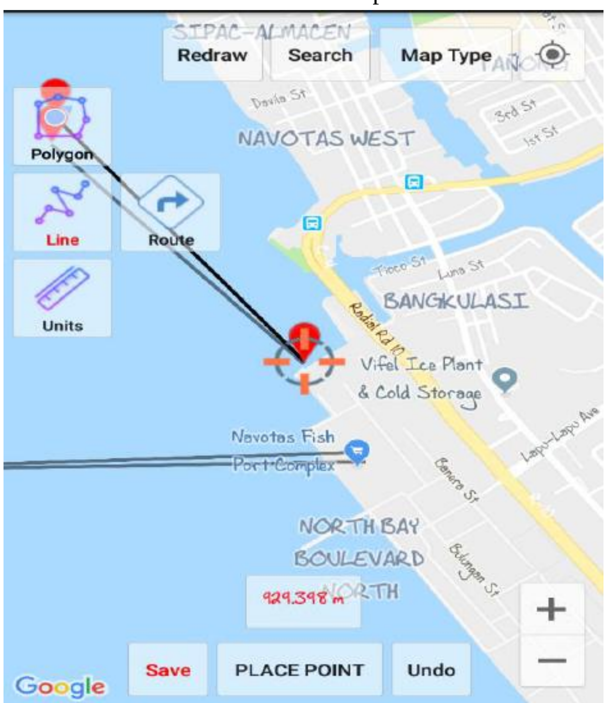

Fig.2: Sampling site. 929.398 m away from the coastline of the Bangkulasi Navotas, Philippines. (we begin the Line transect at Bangkulasi due to boats are docked at Bangkulasi not in Sipac Almacen). Google Earth 2019.

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On figure 2 it shows the sampling site with its line transect from the original point that measures 929.398 from the coast of Bangkulasi Navotas. It should be noted here that we begin our transect line measurement from Bangkulasi not in Sipac Almacen Navotas due to the boats are docked in Bangkulasi Navotas not in Sipac Almacen Navotas.

III. RESULTS AND DISCUSSION

Table 1. Analysis on the physicochemical primary parameters of water sample at Manila Bay Adjacent to Sipac Almacen, Navotas Philippines

Primary Parameters	Unit	DAO 2016-08 Water Quality Guidelines for Primary Parameters	This Study
DO	mg/L	6	3.2
Turbidity	NTU	NA	2.1
PH/Temperature	/0°C	7.0-8.5	7.9@25.0°C
Color	TCU	50	15@7.9
Salinity	NTU	35	35.2

APHA AWWA and WEF 2012/2017 Standard Method for the Examination of Water and Wastewater 22nd and 23rd Edition. F.A.S.T Laboratories. PAB Accredited Testing Laboratory PNS ISO/IEC 17025:2005

Water quality guidelines shall be maintained for each water body classification. (DAO 2016-08). For this purpose, primary and secondary parameters are set to monitor water quality. On table 1, it shows the comparative analysis between the primary parameters set by DAO 2016-08 and from the water sample obtained from the Manila bay adjacent to Sipac Almacen Navotas, Philippines. The dissolved oxygen on the site is much lower than standards set by DAO 2016-08 which can be attributed to few to none at all presence of planktons, algae and other plant-related organisms that dwell in the study site. Lower oxygen count on the body of water indicates that life is threatened in the area and is not in optimal health. Presence of water pollutants will lower the DO considerably as a result of organic matter discharges (Chapman, 1996). Turbidity is an expression of the optical property that causes lightweight materials to be scattered and absorbed rather than transmitted with no alteration in direction or flux level through the sample. The Higher the turbidity the higher is the dissolved substances such as clay, mud, silt and even microorganism that refracts the light(APHA AWWA and WEF 2012/2017). In the study site, a lower value of turbidity indicates that there is a lower density of solid substances and microorganisms that refract the light. The PH level of the water is still within the standards set by DAO 2016-08. While its color is lower than the set standards of DAO 2016-08 which can be attributed to the wastes dumped by informal settlers and even from docked ships and vessels at the coastlines. While its salinity is likewise on the average range of typical saline water having a salinity of 35 ppt.

Table.2: Total coliform count on water sample from the Manila bay Adjacent to sipac Almacen Navotas Philippines.

Sample	Effluent Standards DAO 2016-08 CLASS SB water body category	Multiple tube fermentation technique most probable number (MPN) per 100 mL **TOTAL COLIFORM COUNT**
Sea water from Manila Bay adjacent to Sipac Almacen Navotas	3×10^3	16×10^3

APHA AWWA and WEF 2012/2017 Standard Method for the Examination of Water and Wastewater 22nd and 23rd Edition. F.A.S.T Laboratories. PAB Accredited Testing Laboratory PNS ISO/IEC 17025:2005

On table 2, it shows a highly significant value of coliform present in the water at the study site that can be attributed to the number of informal settlers and their voluminous sewage and domestic wastes dumped directly to the Manila Bay. Fecal coliforms under the group of coliform are used as a mean by which scientists determine if the water is being

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contaminated by sewage. Sewage contains bacteria, viruses and other organisms (collectively referred to as pathogens) that can cause disease in humans such as typhoid fever, hepatitis, gastroenteritis, and dysentery (PEMSEA 2006).

Table.3: Heavy metal assessment in the water of Manila bay Adjacent to Sipac Almacen Navotas, Philippines.

Heavy metal	Permissible limit	Sia Su et.al (2009) bivalve mollusks *Mercenaria sp.* Mollusks Hard clam	This study 2019 bivalve mollusks *Perna veridis* Green mussels
Cd	1.0 [a], 2.0 [b]	1.7214[ab]	Less than 0.005[y]
Cr	0.100	9.54525[ab]	Less than 0.25[y]
Pb	1.5	7.3833[ab]	Less than 0.30[y]
Hg	0.5	No data	Less than 0.30 [y]

[a]Commission Regulation (EC) No/ 1881/2006 by EU; [b]Maximum Levels of Contaminants in Foods (GB 27622012) by MHPRC.

[a] Measured mean total heavy metal concentrations that exceeded the permitted level

[b] Highest mean total concentration for each heavy metal

[y] reporting limit. F.A.S.T Laboratories. Reference. Official method of analysis of AOAC International.

On table 3, it explains that the turbid state of Manila bay resulting from docked ships and fishing vessels, organic and inorganic wastes from anthropogenic sources provides a habitat for sessile organism *Perna viridis*. The means by which filter feeders like mussels acquire their food may also be the same route in which contaminants such as heavy metals enter the body tissues of *P. viridis*. In filter-feeding bivalves like mussels, particulate matters in the ocean are carried in suspension by currents of water pumped through their incurrent siphon, across the gills, then out to the excurrent siphon (Putri, 2012). Results shows that particles greater than 4 µm (including tiny organisms, detritus, suspended sediment, and chemical contaminants) are completely retained by the cirri. They are entangled with mucus then assimilated, or passed through the body without being digested depending on the size, shape, and other physical characteristics of that particle (Jorgensen 1996). This characteristic of *P. viridis* made them resistant to metals and other contaminants.

Although as depicted on the assessment of metals present on mussels, such as cadmium, chromium, lead and mercury is within the permissible limit this does not follow that the water in the area is not contaminated and is conducive for other marine species. This could only suggest that *P. veridis* has a great bioaccumulation capability within an extreme conditions of high level of heavy metals present in the water (Nacua 2018).

Heavy metals such as lead, chromium, cadmium and mercury can be generated as waste from electroplating, nickel plating, smelting, engraving, batteries, sewage sludge, fertilizers, paints, pigments, plastics and waste disposal yard (Alloway and Ayres, 1997; Manahan, 2001; Bagchi, 2004; Cumar and Nagaraja, 2011; Galarpe and Parilla, 2014) while Deposition of Cd may be associated to anthropogenic sources (Velasquez et al., 2002).

However, even if the results of metal assessment in the *Perna viridis* shows that it is within the permissible limit, metal assessment on the sediments of Manila bay shows otherwise on Table 4.

Table.4: Comparative analysis between heavy metal assessment on sediments found in Cansaga bay Cebu, Philippines and Manila bay Adjacent in Sipac Almacen Navotas, Philippines.

Parameters	Cansaga bay Cebu Philippines, 2017	This study 2019
Pb	0.0947	39
Cd	2.2231	0.117
Cr	17.3171	3.64
Hg	Nd	Less than 0.005[y]

[y] reporting limit. F.A.S.T Laboratories. Reference. Official method of analysis of AOAC International.

Like Manila bay, Cansaga bay is located in the metropolitan district and is home to heavily industrialized city in the northeast coastline part of Cebu . Presence of heavy metals are likewise observed in the turbid Cansaga bay, however result shows on table 4 indicates that there is a significant high value of difference on Lead contamination in Manila bay than in Cansaga Bay. This is due to number of docked ships on Manila bay. The high concentration of lead may cause of neurological deficits such as mental retardation in children and kidney disease such as interstitial nephritis to adults. It also contributes to hypertension and cardiovascular disease to the consumers in the coastal areas after long term consumption (Hossen 2015). Although cadmium and chromium present in Cansaga bay display a higher value than Manila bay this can be attributed to the type of industry that dumps cadmium and Chromium specifically industries that uses and burn coal to produce steam (Kimbrough1999) as a waste product in the bay. Mercury assessment results present in Manila bay indicates that it is within the permissible limit.

Table.5: Heavy metals in Manila bay adjacent to Sipac Almacen Navotas, Philippines compared to threshold quality guidelines from different sources

SQG	HEAVY METALS mg/kg				Reference
	Cd	Cr	Pb	Hg	
Manila Bay	0.117	3.64	39	less than 0.005[y]	This study
TEL[a]	0.6	37.3	35	Nd	Macdonald et.al 2000
ERL[b]	5	80	35	Nd	Macdonald et.al 2001
LEL[c]	0.6	26	31	Nd	Macdonald et.al 2002
MET[d]	0.9	55	42	Nd	Macdonald et.al 2003
NOAA ERL[e]	1.2	81	47	Nd	NOAA 1999
HONGKONG ISQG-low[f]	1.8	80	75	Nd	ANZECC 1997

Threshold Sediment Quality Guidelines from Different Sources. Galarpe et.al. (2017)
Threshold effect level, [b]effects range low, [c]lowest effect level, [d]minimal effect threshold, [e]National Oceanic and Atmospheric Administration, [f]Hong Kong-interim sediment quality guideline.

With respect to the threshold quality guidelines of sediments from different sources by Galarpe et.al (2017), the heavy metal assessment of sediment from this study is under the permissible value. Although they exhibit significant value than the one exhibited by the results in the *Perna viridis*, it is still within the permissible range. The mercury range of the sediment is still on the permissible value.

IV. CONCLUSION

Perna viridis in Manila Bay was observed for not having any serious deformities and signs of visceral necrosis with its gross morphology intact and concurring with published descriptions and values. It has been assessed that it has tolerable levels of Cd, Cr, Pb, and Hg with reference to international/national standards. With reference to the previous similar study done in Cansaga Bay Cebu, Philippines, this study obtained a lower total Cd, Hg, Pb and Cr both in sediments and the P. viridis but higher in terms of total coliform level in reference to DAO 2016-08. This study is the first attempt to measure total Hg in P. viridis in Manila Bay but the concentration obtained is below the detectable limit. It is recommended to have a follow-up monitoring of heavy metals in fish and shellfish commodities in Manila Bay as the influx of industrial and anthropogenic wastes to the bay is becoming extensive (DAO2016-08 table 2-3).

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DECLARATION OF CONFLICT OF INTEREST

I the correspondent author declare that there is no conflict of interest for this paper entitled Assessment of Heavy Metals in Philippine Green Mussels *Perna viridis* and Level of Coliform on Manila Bay Adjacent to the Coastline of Sipac Almacen, Navotas Philippines. And I hereby address to the set rules and regulations mandated by this publication.

DATA AVAILABILITY (SUPPLEMENTARY MATERIALS)

Conducted and verified results by FAST LABORATORIES, Cubao Quezon City Philippines be submitted on a separate file on a pdf format.

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