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| RESEARCH ARTICLE

Analysis of Heavy Metal (Hg, Pb, Ni) Content in Marsh Clam, *Polymesoda expansa* Collected from Butuan Bay, Philippines

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| ABSTRACT

Butuan Bay is an amalgamation of resources and wastes, with the Agusan River as its main river source. Multiple industrial operations near the area make it susceptible to pollution, thereby affecting the aquatic organisms and the people who depend on the bay for food and livelihood. Thus, the main objective of the study was to determine the Mercury (Hg), Lead (Pb), and Nickel (Ni) concentration in the flesh of Marsh clam (*Polymesoda expansa*) in different mangroves wetlands across Butuan Bay. Heavy metal concentration was assessed using cold vapor atomic absorption spectroscopy for Hg and Atomic Absorption Spectrophotometry for Pb and Ni. The results revealed that Hg and Pb concentrations were below the detection limits (BDL), while Ni concentrations were within the tolerable limits established for bivalves. Human health risk via the consumption of *P. expansa* was calculated using the estimated daily intake (EDI) and Target Quotient Hazard (THQ). Results show that the EDI of Ni exceeded the oral reference dose, but its THQ was below the standard THQ value. In conclusion, the consumption of *P. expansa* does not pose any adverse health effects on human health. Still, further investigation and monitoring of the mangrove ecosystems are encouraged to secure the environment's health and the communities that depend on it.

| KEYWORDS

Biology, heavy metal contamination, heavy metal analysis, human health risk, estimated daily intake, target hazard quotient, *Polymesoda expansa*, Butuan Bay, Philippines

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1. Introduction

Mining is one of many economic activities frequently used in several developing countries (Ugya et al., 2018). Despite these operations being one of the main driving forces for economic growth, mining activities (even after cessation) still risk pollution in the environment. Activities such as loss of biodiversity, soil contamination, air pollution, natural land degradation, and surface and groundwater pollution produce excessive waste leading to decades of environmental harm. Mining effluents from these activities (mainly heavy metals) are pollutants that have the potential to accumulate in aquatic organisms that enter the human food chain (Fatima et al., 2014). Specifically for humans, the contraction of heavy metals may be possible through drinking water or through the consumption of food. The United States Food and Drug Administration (2015) states that small traces of heavy metals carry the risk of organ and organ system poisoning in humans when inhaled or ingested. Furthermore, the presence of such risks seems to be unrecognized in numerous rural areas, especially ones located on coastlines or ones which use fishing as the primary source of income and food.

Several studies show a high great of heavy metal contamination in Butuan Bay. The accumulation of mercury in Agusan River was observed in water cabbage (*Pistia stratiotes*), which exceeded the permissible limits established by WHO (Demetillo & Goloran, 2017). Fish samples collected from Barangay Buhangin, Barangay Pagatpatan, and Barangay Dahekan exhibited levels of cadmium, chromium, copper, lead, and nickel that surpassed safe limits. Sediment samples from the same location showed excessive limits of mercury, chromium, and nickel (Cabuga et al., 2019).

A study conducted by Cabuga et al. (2016) in the lower Agusan River basin found significant levels of lead, copper, mercury, and cadmium in the muscles of Pigok (*Mesopristes cancellatus*). Sediment analyses revealed that nickel had the highest mean, with mercury and cadmium concentrations exceeding the recommended safe limits set by FAO and WHO. They had also discovered levels of heavy metal concentration in the flesh of *Guama Johnius borneensis* and sediments from the same location. In the aforementioned study, levels of total mercury, lead, cadmium, and nickel content appeared to have exceeded the standard allowable limits set by USEPA and FDA. In addition, there has been existing research on bivalves ingesting heavy metals, such as *Polymesoda erosa*. The results of the samples gathered from Barangay Pagatpatan, and Barangay Camagong indicated that mercury, nickel, and chromium concentrations exceeded the allowable limits for sediments, while lead levels were above the standards for P. erosa muscles (Elvira et al., 2016).

Among the studies conducted in the Agusan River basin, none of them has utilized marsh clam (*Polymesoda expansa*) as an indicator of heavy metal accumulation, which is a common food source in communities near Butuan Bay. Due to the bivalve's filter-feeding mechanism, it has been widely used as a bioindicator in Malaysia to assess water quality (Yahya et al., 2018). Hence, this study focuses on utilizing *P. expansa* as a possible bioindicator of heavy metal accumulation in Barangay Pagatpatan and Barangay Masao. They were chosen as sampling sites because of their location near the mouth of Agusan River, and recent research suggests heavy metal contamination in their waters (Cabuga et al., 2019; Elvira et al., 2016).

Hence, this study aims to achieve the following objectives: to determine the concentration of mercury, lead, and nickel in the flesh of Marsh clam in selected mangrove wetlands of Butuan Bay; to analyze and compare the expressed levels of Hg, Pb, and Ni contained in the flesh of *P. expansa* with that of the maximum permissible limits according to International Guideline values; and to estimate whether the consumption of P. expansa poses any adverse health hazards.

2. Literature Review

Various aquatic organisms such as clams feed by trapping food particles in their bodies, making them potential bio-accumulators of heavy metal elements. Several studies have expressed concern about the water quality of the Agusan River and its tributaries, as their results revealed significant levels of trace elements in the flesh of fish and clams. Hence, in this literature review, studies about lead, mercury, and nickel content analysis in the muscles of Marsh clam, Polymesoda expansa are the focus of the discussion.

2.1 Distribution of P. expansa in the indo-pacific region

According to Sandilyan & Kathiresan (2012), mangroves serve a great socio-economic and ecological value as a center for tropical marine biotopes. Mangroves are also one of the world's richest depositories of genetic and biological diversity. Moreover, 80 % of the global fish catch relies on mangroves, and 90 % of marine organisms spend part of their life in this ecosystem.

Dispersed widely across the Indo-Pacific, mainly in the tidal flat of Southeast Asia, is the mangrove clam *Polymesoda expansa* (Mousson 1849) which is known to be a deep burrowing bivalve. Mussels, clams, and oysters, which are marine edible bivalves, are widely distributed and are a feasible fishery along with the Goa, Maharashtra, and Karnataka coast of the west coast of India. *Polymesoda erosa*, *P. bengalensis*, and *P. expansa* are the three species of the mangrove clams that were reported from the Indo-Pacific region.

The distribution of *P. expansa* is noted to have a wider, rather overlapping distribution in the Indo-Pacific from North to Vietnam; India to Vanuatu; and South to Eastern Java. It has been hypothesized that *P. expansa* is less tolerant of colder waters of the Northern and Southern extremities of its distribution. Also, this species is also reportedly seen in man-made prawn ponds. In Sarawak, the Corbiculidae family was recorded from all groups where *P. expansa* is one of the often abundant species found after *P. erosa* in the mudflat habitat (Yahya et al., 2018).

Regardless of their abundance, economic significance, and food potential, mud clams have received very minimal attention. Research on *Polymesoda expansa*, which includes population, reproduction aspect, and heavy metal accumulation, has not been well explored. Hence, this study utilizes *P. expansa* as a possible bioindicator of heavy metal accumulation.

2.2 Bivalve mollusk as a bioindicator in assessing ecosystems' health

The conventional method of monitoring metals is by evaluating the concentration of heavy metals in water and sediments. However, this method does not offer accurate and substantial information, which has greatly misled efforts that aim to regulate heavy metal contamination. To solve this issue, researchers have looked into using benthic organisms as an alternative (Waykar & Deshmukh, 2012). Most bivalves feed by trapping microscopic food particles in the water around them in a process called filter-feeding. This is done by propelling water from the posterior ventral region through the inhalant siphon, and the water passes through the gills and is ejected through the exhalant siphon (Krishnakumar et al., 2018). Several studies have utilized bivalve molluscs as bioindicators and biomonitors because they adhere to the characteristics of a good bioindicator. Aside from being a filter-feeder capable of accumulating metals from food and water, they are somewhat sedentary, regionally abundant, long-lived, and have adequate tissue mass for analysis (Waykar & Deshmukh, 2012).

Dabwan and Taufiq (2016) showed the differing capacities of *Anadara granosa* and *P. expansa* in accumulating heavy metals. *A. granosa* had a tendency to accumulate a higher concentration of nonessential metals (Cd and Pb) compared to *P. expansa*, where it had a higher tendency to accumulate the essential metals Cu, Zn and Fe. In a transplantation experiment on oysters and clams by Hédouin et al. (2011), the oyster *Isognomon isognomon* managed to reach Cr and Cu concentrations found in resident organisms in 100 days, while the edible clam *Gafrarium tumidum* achieved the same with Co, Ni, and Zn. Thus, the uptake kinetics of bivalve mollusks signifies its importance as a bioindicator.

In summary, bivalve mollusks are often used as bioindicators due to their filter-feeding mechanism. Such a mode of nutrition leaves them susceptible to accumulating trace metals. Their long lifespan and sedentary nature enable them to amass trace metals over a long period of time, which can be easily analyzed given their ample flesh size. Bivalve mollusks are regionally abundant, and they have significant uptake kinetics. Hence, they are good bioindicators for evaluating the ecosystem's condition.

2.3 Nature of heavy metals

Heavy metals are regarded as naturally occurring and potentially toxic resources in the environment. However, its constant use in rapid industrial activities resulted in high concentrations of these metals, exposing humans to such levels that can lead to adverse health effects (Mishra et al., 2019). The toxicity may vary in the type of heavy metal, exposure dose, frequency and duration of exposure, and the characteristics of the person. Nonetheless, these metals pose harm in many cases, and thus, extensive studies focused on the properties and toxicities of these metals.

2.3.1 Nature of Mercury

Mercury is widely assumed to be the most toxic heavy metal due to causing several major health incidents around the world (Bernhoft, 2012). Its pure state often referred to as "elemental" or "metallic" mercury, naturally exists in the Earth's crust (Agency, n.d.). Mercury concentrations in the air are usually from anthropogenic activities such as coal smoke or gold mining. Mercury can also be found in water due to runoff from mining waste or mercury-contaminated rain (Zahir et al., 2005). Furthermore, mercury may accumulate in aquatic sediments as well in filter-feeding organisms that dwell in this environment. Thus, mercury exposure in humans mainly comes from inhalation or ingestion of seafood.

Robin Bernhoft's journal article titled "Mercury Toxicity and Treatment: A Review of the Literature" discusses the various forms of mercury and their effect on the body. Specifically, Bernhoft discusses the sources of mercury exposure, its toxicity in different compositions, and the clinical presentations associated with mercury exposure in the human body. Upon reviewing, Bernhoft implies the lack of awareness regarding mercury-induced symptoms such as fatigue, depression, and anxiety. The capability of mercury traces reaching different parts of the body widens the range of the symptoms it causes. However, diagnosis may become difficult as standardized tests do not necessarily confirm that mercury exposure is causing the symptoms presented. This brings upon the urgency for research advancement on mercury since cases of mercury exposure are significantly high (Bernhoft, 2012).

2.3.2 Nature of Lead

Lead is a heavy metal that is denser than most common materials. It is soft, malleable, and has a relatively low melting point (Boldyrev, 2018). Lead's highly usable properties made it valuable and important in the innovations of many things in the modern world. Furthermore, it is evident that its flexibility in use for many purposes made lead a sought-out resource in many countries, resulting in frequent lead extraction from the environment. Despite this, however, lead is non-biodegradable, resulting in lead waste from mainly industrial activities accumulating and polluting the environment in high concentrations.

It is a highly poisonous metal (whether inhaled or swallowed), affecting almost every organ and system in the human body. At airborne levels of 100 mg/m3, it is immediately dangerous to life and health (Administration, 2015). An in-depth review was written by D.A. Gidlow in 2015 that tackled the toxicity of lead in the human body. This review serves as an update to the previous one in 2004 since "there have been further developments with regard to safe exposure levels for lead workers". To summarize, the author discusses the range of complications an individual may experience when exposed to varying levels of lead over varying periods of time. One may be at risk of neurological ailments, carcinogenic effects, renal failure, and hypertension, to name some listed in the review. Women might also be at risk of reproductive complications and developmental effects on their children.

2.3.3 Nature of Nickel

Nickel is one of many commonly found natural materials among most metals and elements in nature. Whether found in the atmosphere, in the soil, or in the water, this heavy metal naturally occurring in the crust comes in various forms and compositions (Zambelli & Ciurli, 2013). Nickel also contains its own unique chemical and physical properties that make it flexible and useful for many modern applications in the industry. Paired with its abundance, nickel usage has been frequent. In addition, shortages of nickel deposits rarely occur, yet the rising demand for it has resulted in excessive reaction to keep profitable production (Everhart, 2012). In a 2020 Nickel Data Report conducted by the United States Geological Survey, the U.S. alone has consumed between 90,000-110,000 tons of nickel yearly for the past 5 years. Several cases of nickel pollution and poisoning have been documented all around the world, possibly stemming from excessive mining and constant use.

In a chapter of a journal written by Barbara Zambelli and Stefano Ciurli (2013), they discussed the toxicity of nickel towards humans and plants, indicating that despite the essentiality nickel has in plants and in bacteria, its toxicity should still be recognized, especially towards humans. When high in concentration, nickel exposure in humans may occur through ingestion, inhalation, or skin absorption. Nickel-induced carcinogenesis and allergies were emphasized in this chapter, as they were defined to be the "most diffuse hazardous effects caused by nickel exposure." Additionally, chronic nickel exposure may lead to several respiratory, cardiovascular, and kidney complications, amplifying the danger of this metal to human health.

2.4 Related Studies

Given that heavy metals are known toxic pollutants that are being heavily used for industrial, agricultural, domestic, and technological purposes (Tchounwou et al., 2012), many researchers are concerned about its bioaccumulation in sediments and in aquatic life, with many communities depending on fisheries for profit and for consumption. Hence, a great number of studies are published aiming to determine the heavy metal concentration in sediments, soils, and organisms collected from locations speculated to be contaminated with such.

A study conducted by Samaniego et al. (2020) aimed to measure mercury content in soil and sediments in Honda Bay coast and Tagburo river, areas with close proximity to an abandoned mercury mine in Palawan. Results show that mine waste calcines contained mercury concentrations ranging from 52.7–924.2 mg kg−1. Other samples collected at Tagburo river and Honda Bay coast were also presented with mercury levels similar to those of the mine waste calcines, exceeding limits established worldwide. The researchers also pointed out the great range increase in mercury levels in Honda Bay compared to the last recorded measurements 20 years ago.

The results from this study justify the concern for mercury contamination in surrounding areas of other listed abandoned mines in the country. However, having data regarding mercury levels from soil and sediments are insufficient; further studies that can target mercury content in biota and in the air are necessary for a human health risk evaluation.

Similarly, the study at hand also explores the probability of high mercury content in areas suspected to be contaminated with the said pollutant. The difference between the two studies, however, lies in the source of interest. The current study will focus on the flesh of mud clam in the area as opposed to soil and sediments from the other study.

Another study conducted by Solidum et al. (2013) aimed to determine lead, cadmium, and chromium concentrations in the head, meat, and internal organs in samples from 9 fish species sold in Metro Manila wet markets. This study made use of the Flame Atomic Absorbance Spectroscopy to analyze heavy metal content in the samples. Using qualitative and quantitative analyses and using US-EPA and FDA guidelines as bases of standard, the researchers were able to find exceeding levels of cadmium and chromium in most fish samples. Lead content, on the other hand, was still within acceptable limits in 8 out of 9 fish species sampled in the study. Additionally, the study found no significance between the head, meat, and internals of the fish samples, meaning that the intake of any part specified in the study would result in the same amount and effect of heavy metal intake regardless of the part.

Having fish from wet markets in a densely populated area as the species of interest, along with significant results in this study, intensifies the need to look into heavy metal contamination. However, findings from this study cannot specify any probable and specific sources of the contamination. This study would require extensive research in order to pinpoint areas contaminated and accumulated with said heavy metals.

Similarly, this study and the current study are focused on heavy metal content in the flesh of filter-feeding animals. With this, the former is only limited to fish species when a wider array of aquatic animals are available in wet markets. It also encompasses a wide variety of fish samples from different sources, making it difficult to exact a location suspected of contamination.

Lastly, a study conducted by C. Cabuga et al. (2016) aimed to determine mercury, lead, copper, cadmium, and nickel content in samples collected in three areas along the lower Agusan River Basin. From this study, the researchers were able to discover levels of heavy metals in both fish and sediment samples, with the lead having the highest concentration in fish muscles among all heavy metals. In sediments, all heavy metal concentrations were found to be within established limits except for nickel. In conclusion, in this study, researchers emphasized the needed concern for the nickel, lead, and cadmium content in the samples collected. Furthermore, the researchers argued that even if levels are within tolerable limits, the possibility of heavy metal bioaccumulation should still be considered.

This study shows that heavy metal contamination in fish and in sediments is evident in places speculated to have such contamination. Among the heavy metals targeted, nickel concentration ranks the lowest in fish muscles, suggesting that nickel-induced teratogenic and carcinogenic toxicity risks are not yet alarming. The high concentration of nickel in sediments, however, could be an influencing factor in the heavy metal contamination in fish. Therefore, further studies can be conducted to possibly trace its pathway in accumulation both in fish and in sediments.

This study and the current one are similar in terms of using aquatic animals as target sources for contamination and the location being Butuan. Both studies also aim to quantify nickel content in these areas along with other heavy metals. Although in view of the species of interest, the present study aims to use a different filter-feeding animal. Fish are widely used in this field of study, knowing that they are considered good bioindicators (C. Cabuga et al., 2016). However, bringing in a new species that is not commonly used could give new insight as to how different organisms are affected by heavy metal contamination.

2.5 Human health risk associated with heavy metal (Hg, Pb, Ni) via Molluskan species/shellfish

Mollusks are capable of bioaccumulating traces of heavy metals due to their filter-feeding mechanism. This poses a problem as they are considered valuable sources of food. Fortunately, authorities such as the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) have established limits pertaining to the amount of heavy metal concentration that is deemed safe for an individual to consume.

Mercury pollution in the Philippines mostly comes from mined deposits and gold extraction (States, n.d.). Approximately one kilogram of Hg is used per week in extracting gold from ores (States, n.d.). This and mining operations have amounted to traces of mercury running into the river systems. Perelonia et al. (2017) had detected Hg concentrations in oyster and mussel samples that ranged from undetected to 1.1063 mg/kg and undetected to 0.1271 mg/kg, respectively. Some oyster samples in eastern Bulacan failed to adhere to the established limits.

Research on *Corbicula fluminea*, conducted in Laguna de Bay, exhibited EDI amounts that exceeded the WHO/FAO maximum tolerable concentration (MTC) of 0.30, suggesting potential health risk effects (de la Cruz et al., 2017). The target quotient (THQ) is an integrated risk index proposed by USEPA to assess the non-carcinogenic risks of metals in contaminated seafood (Ahmed et al., 2015) by taking the ratio of the ingested amount of the contaminant with a standard reference dose. The THQs of the aforementioned study were only 0.20 and 0.50 for an average and heavy clam consumer, respectively. Based on the guidelines purported by USEPA, there were no adverse non-carcinogenic risks in the study since the THQ values are below the threshold value (THQ=1.00). Another study in the muscles of selected shellfish (*Portunus reticulatus*, *P. segnis*, *P. sanguinolentus*, *Scylla olivaceae*, *Penaeus monodon*, and *P. indicus*) suggested a significant difference ($p > 0.05$) in the metal concentration among species. THQs were all below 1; however, calculations of the Cancer Risk index indicated that Pb caused the greatest cancer risk along with Cd (Saher & Kanwal, 2019).

In the study conducted by (Abdallah, 2013), the highest concentration of Ni was measured at 2.832 lg/g in *V. rhomboids*, which is within the permissible limits. Humans are often exposed to Ni through food, natural sources, and food processing. The oral intake of Ni that is considered allowable for humans is 300–600 lg/day. A study that utilized *Polymesoda erosa* conducted in Butuan Bay, Philippines, showed alarming results on the level of Ni in sediments, and Elvira et al. (2016) suggested it needs to have a local intervention to regulate the mining activities along with in Butuan Bay, such operation of San Roque Metal Incorporated located in Tubay, Agusan del Norte.

In conclusion, there is no guarantee that the shellfish one is eating is contaminated with traces of heavy metals. If it is, there is no assurance that its heavy metal concentration is within the permissible limits. Such cases depend on the pollution levels of the location from which the shellfish was obtained. Therefore, the present study aims to collect samples from areas that are known to be polluted.

3. Methodology

3.1 Study Area

The study was conducted in two different mangrove ecosystems of Butuan Bay, namely, Pagatpatan and Masao mangrove wetlands.

Figure 1. Location of two different mangrove ecosystems of Butuan Bay.

3.2 Collection Procedure

Individuals of Marsh clams per station were randomly gleaned for the determination of mercury (Hg), lead (Pb), and nickel (Ni) concentrations. Their identities were validated by a clam specialist to confirm that they are indeed Polymesoda expansa. The collected samples were placed in a plastic container with water to keep the specimens alive while transporting them to the laboratory. Upon arrival at F.A.S.T. Laboratories, Cagayan de Oro City, the samples were sorted and placed in a laboratory set-up favorable for the clam to survive before the analysis. This set-up allows the clams to release ingested sediments before the analysis.

3.3 Digestion of Flesh Sample

Acid digestion of flesh samples was conducted using nitric acid (NHO3) and sulfuric acid (H2SO4).

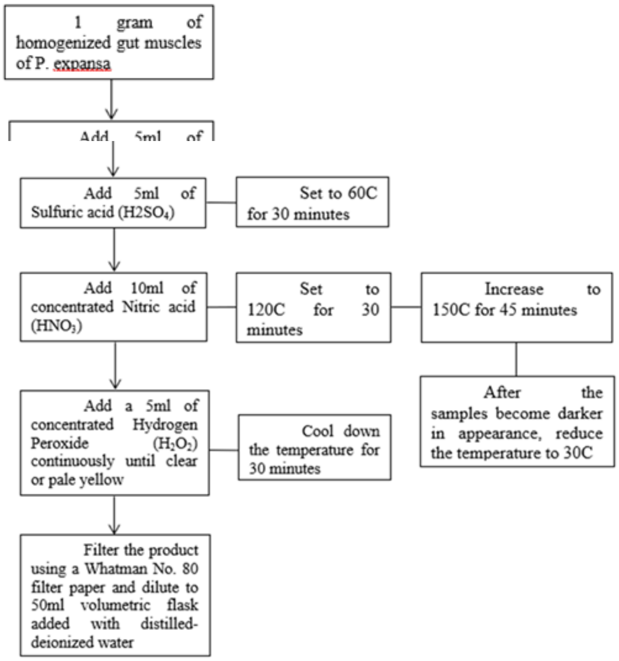

Figure 2. Framework of muscle digestion based on the official methods of analysis of AOAC International, 19th Edition (2012).

3.4 Sample Analysis

The digested flesh samples were analyzed using cold vapor AAS for Hg and atomic absorption spectrophotometry for Ni and Pb concentrations. This heavy metal analysis was conducted at FAST Laboratories, Cagayan de Oro City.

3.5 Data Analysis

3.5.1 Toxicity Risk

Among the several established methods used to estimate the potential risk of toxic metals on human health, the USA Environmental Protection Agency (USEPA 1989) promotes and utilizes the target hazard quotient (THQ) and hazard index (HI). These indices were recognized for the evaluation associated with the ingestion of metals through the consumption of contaminated foods (Hough et al., 2004; Chary et al., 2008). As defined by the USEPA (1989), the maximum tolerable daily intake of a specific metal that does not result in any deleterious health effects is expressed below:

$$THQ = \frac{EFr \times ED \times IR \times MC}{RfD \times BW \times AT} \times 10^{-3}$$

Where EFr is the exposure frequency (365 days year^{-1}); ED is the exposure duration (70 years) equivalent to the average human lifetime; IR is the food ingestion rate (g person^{-1} day^{-1}); MC is the metal concentration of samples (mg kg^{-1}, wet weight); RfD is the oral reference dose (mg kg^{-1} d^{-1}); RfD is based on 0.02 mg kg^{-1} day^{-1} for Ni (USEPA IRIS, 2005) adopted from the study of Elvira et al. (2020); BW average body weight of a Filipino (65 kg) adopted from the study of Molina (2014); AT is the averaging time for non-carcinogens (365 day/year^{-1} × number of exposure years, assuming 70 years).

If the THQ value is less than one, the exposure population is unlikely to experience any adverse health hazard. If the THQ is equal to or higher than one, there is a potential health risk (Wang et al. 2005), and related interventions and protective measurements should be taken.

3.5.2 Estimated Daily Intake (EDI)

The estimated daily intake (EDI) of metals depends on both metal concentration in the clams and the rate of consumption of the fishing households across stations of Butuan Bay. The equation to calculate the EDI based on Song et al. (2009) is expressed below:

$$EDI = \frac{IR \times MC}{BW}$$

Where EDI is the estimated daily intake of metals through consumption of clam by the fishermen in Masao and Pagatpatan in grams per daily basis; IR is the estimated daily consumption of the flesh of the clam (g/person/day); MC represents the concentration of heavy metals in the clam (µg/g for fresh weight), and BW is the bodyweight of an adult (65 kg). The oral reference dose (RfD) value for nickel is 0.02 mg kg^{-1} day^{-1} (USEPA, IRIS), which is used to compare the EDI of the fishermen. This method was adopted from the study of Zhao et al. (2012) and Elvira et al. (2020).

4. Results and Discussion

4.1 Heavy Metal Concentration in the Flesh of P. exapnsa

Table 1. Heavy Metal Concentrations in the Flesh of *P. expansa*

Heavy Metal (mg/Kg)	Pagatpatan	Masao	Standard (mg/kg)	Significant Difference (P < 0.05)
Hg	< 0.01	< 0.01	0.5	-
Ni	2.38 ± 0.83	1.73 ± 0.66	80	0.3489
Pb	< 0.25	< 0.25	0.2	-

Laboratory results show that the estimated mean for Hg and Pb concentration in the flesh of *P. expansa* was below the respective detection limits established by utilizing different instruments for measuring each heavy metal. This implies that Hg and Pb contamination in mangrove ecosystems in Pagatpatan and Masao are unlikely due to the small amount observed from the results. Still, said contamination is possible due to ongoing anthropogenic activities such as illegal logging and kaingin practices that result in soil erosion and siltation of the rivers.

Mining operations in Mount Diwata use mercury, cyanide, and explosives, which have serious direct effects on the environment and indirect effects on the inhabitants of the areas. High levels of Hg were found near Diwalwal and some other mining sites, and though dispersion patterns suggest that dissolved Hg is rapidly lost and diluted, a reservoir of Hg may accumulate and cause a potential long-term pollution issue (Breward, 1996).

As for lead, it is a commonly sought-out heavy metal due to its usable properties, resulting in frequent lead extraction from the environment. The common source of lead is mining and burning of fossil fuels (Elnabris et al., 2013). However, lead is non-biodegradable, and as such, accumulation is inevitable if continual disposal of lead waste from industrial activities prevails over time.

On the other hand, results reveal that Ni concentration is evident with mean values of 2.38 ± 0.83 mg/kg in Pagatpatan and 1.73 ± 0.66 mg/kg in Masao. This level of concentration is well within limits imposed by the FAO/WHO (1984). The higher concentration in Pagatpatan is possibly due to its proximity to the mouth of the Agusan River Basin. According to Velasco et al., 2016, Pagatpatan comprises one of many areas in the Lower Agusan River Basin ridden with anthropogenic activities from industrialization. Such Ni content may be due to discharges from industrial, domestic, and agricultural sewages, leading to its accumulation in sediments and in marine life with time (Cabuga et al., 2016). Still, there is no significant difference in Ni concentration between the two areas. This possibly implies that both areas, Pagatpatan and Masao, are under the same environmental conditions to have accumulated such levels of Ni content in their Marsh clams.

4.2 Measures of Human Health Risk

Due to community health protocols, the researchers were unable to conduct a survey that would provide the needed data for calculating the THQ, such as the food ingestion rate (IR) of an individual in a day. As such, data from related studies were utilized. The IR value of 15.8 g/person/day was adopted from Laurenti (2007), which is within the range of high consumption rates in the study of Elvira et al. (2020).

4.2.1 Estimated Daily Intake (EDI)

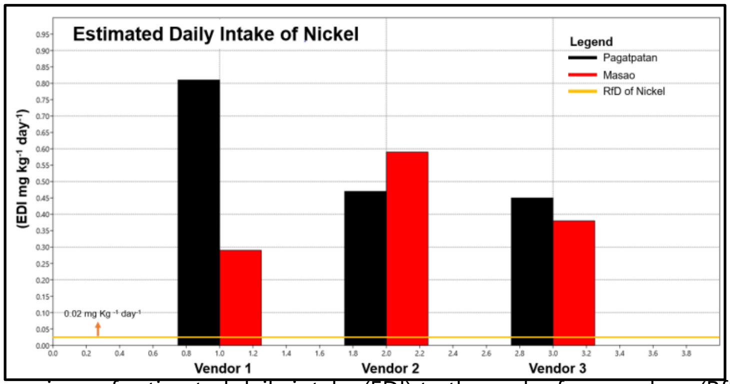

Figure 3. Comparison of estimated daily intake (EDI) to the oral reference dose (RfD) of Nickel.

The estimated daily intake (EDI) of Ni measures the amount of Ni content consumed through daily ingestion of Marsh clam flesh. It is then compared to the oral reference dose imposed by the United States Environmental Protection Agency, Integrated Risk Information System (USEPA, IRIS), in assessing the health risk associated with the contamination of Ni in humans. EDIs of Ni were revealed to be at 0.809 mg kg^{-1} day^{-1}, 0.469 mg kg^{-1} day^{-1}, and 0.455 mg kg^{-1} day^{-1} in Pagatpatan and 0.287 mg kg^{-1} day^{-1}, 0.598 mg kg^{-1} day^{-1}, and 0.377 mg kg^{-1} day^{-1} in Masao.

Despite that Ni concentrations are within tolerable limits imposed by the FAO/WHO (1984), Figure 3 shows that the EDI of Ni far surpasses the standard reference dose, which is 0.02 mg/kg per day. This suggests that human bodies are more susceptible to Ni contamination than marine life, as limits imposed on bivalves were far greater than the limits imposed on humans. With this in mind, even small traces of Ni concentration could already raise a concern or risk the health of many human bodies.

4.2.2 Target Hazard Quotient (THQ)

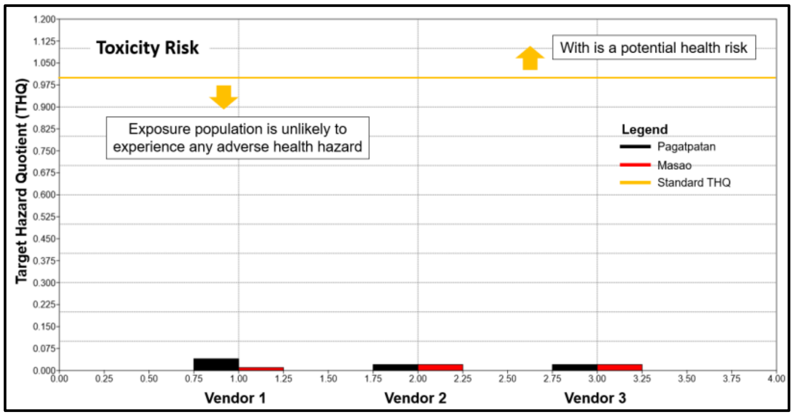

Figure 4. Target hazard quotient (THQ) on heavy metals of consumers.

The target hazard quotient (THQ) is the ratio of exposure to a toxic element and the reference dose, which is the maximum at which no adverse health effects are expected. A THQ value less than one implies that the exposure population is unlikely to experience any adverse health hazard. On the other hand, a THQ value equal to or higher than one would mean that there is a potential health risk (Wang et al. 2005), and related interventions and protective measurements should be taken.

The THQ of Ni across the sampling stations appeared to be less than one, suggesting that the exposure population is unlikely to experience any deleterious effects from the consumption of *P. expansa*. However, it should be noted that the THQ value is not only attributed to the heavy metal content in the flesh of *P. expansa* but it is also affected by the exposure frequency (EFr) and the ingestion rate (IR) of an individual to the said clam.

5. Conclusion

The recorded heavy metal concentrations are well within the tolerable limits in bivalves. It also seems that the calculated estimated daily intake of nickel exceeded the imposed oral reference dose for nickel, and THQ is below 1. Based on these findings, the researchers conclude that Marsh clams collected from mangrove wetlands in Pagatpatan and Masao currently do not pose adverse health effects on human health. However, the EDIs of nickel does raise some concern as it far exceeds the RfD limit.

Therefore, regular monitoring of these environments will still be necessary as the areas are within numerous anthropogenic sites. While some operations have been discontinued, the discharge of contaminants from these abandoned structures into aquatic ecosystems could still possibly occur and will threaten the environment and the community in the long-run. As these ecosystems are essential to the residents who rely on fisheries for their livelihood, efforts in ensuring the safety and well-being of these ecosystems must be prioritized and maintained to support the rural communities that reside near these kinds of environments to protect them from any economic or health-related issue in the future.

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