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TRACES OF HEAVY METALS AND HEALTH RISKS OF FRESHWATER ASIAN CLAM (*CORBICULA FLUMINEA*) CONSUMPTION FROM TWO SITES IN LAGUNA LAKE, PHILIPPINES

ROMEO C. PATI* AND ROSALINDA G. BRASOS

College of Agriculture, University of Rizal System-Tanay Campus, Philippines

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ABSTRACT

The concentration of selected heavy metals and human health risks associated in consuming clam with traces of As were investigated. The clams were harvested in two clam habitats located in the eastern and central bay areas of Laguna Lake. Concentrations of Pb and Cd in pooled clam soft tissues were determined by Ashing Acid Digestion and Inductively Coupled Plasma Atomic Emission, respectively. The concentration of As was determined through Hydride Vapor Generation and Atomic Absorption Spectrometry. The health risks in clam consumption due to traces of As were estimated using the methodology developed by USEPA (2004). Traces of Cd and As at varying concentrations were detected in the pooled clam tissues in the study sites. Arsenic in clam tissues had concentrations ranging from 1.4 to 1.6 mg*kg^{-1} in Barangay Bangad, Binangonan Rizal. Cadmium had lower concentrations in clam tissues, equal to 0.04 mg*kg^{-1} in clam sites near Barangay Punta, Jala Jala, Rizal. Target hazard quotient estimates indicated that the concentrations of As in clam are below the values that can cause adverse non-carcinogenic health effects due to long-term consumption. On the other hand, the lifetime cancer risk estimates at 30% and 100% concentrations of inorganic As were all outside the recommended ranges of regulatory agencies abroad. The data indicated that clam consumers had the potential of developing a cancer if the consumption was high, frequent, and lifetime.

KEY WORDS : Clam, Heavy metal, Health risks

INTRODUCTION

Environmental degradation, especially in the aquatic ecosystems, is a growing concern globally and locally. Contaminants like chemicals, nutrients and heavy metals are discharged naturally, accidentally, or intentionally in different water bodies in the Philippines. Heavy metal contamination of aquatic ecosystems has been a problem in urban centers in many parts of the country (Molina *et al*., 2011). Laguna de Bay, a significant natural resource located in the country's center of urban and industrial development, is a recipient of continued contamination of heavy metals. The mixed discharges of heavy metal wastes from anthropogenic activities and natural occurrences contribute to the increase of concentrations of these pollutants in the lake, most especially in the sediments where they are ultimately deposited. Lead, cadmium, and arsenic have been detected in the sediments in many parts of the lake (Hallare *et al*., 2005). These contaminants can persist for years and may accumulate in the sediments and have the potential to adversely affect aquatic species as well as humans (Amisah *et al*., 2010).

Since heavy metals are non-biodegradable, once they enter the aquatic environment, they can be accumulated through metabolic and bio-absorption processes (Bervoets *et al*., 2001). This can trigger problems in both aquatic species and humans. Lead for example is known to inhibit enzymes essential in the biosynthesis of hemoglobin in humans and this can lead to anemia (Flora *et al*., 2012). Waterborne heavy metals such as cadmium decrease the fecundity in freshwater snails at low level exposure and cause death of embryo at high level exposure (Ansaldo *et al*., 2009).

Most often, the recipients of the pollutants that are deposited in the sediments are the bivalves such as clams. These species burrow into the sediments and feed from the water column and the substrate (McMahon, 2002). They filter suspended particles from the water, hence have the tendency to absorb more pollutants than other aquatic species. Clams have been used as bio-indicators and have also been used for toxicity testing (Paller *et al.*, 2013) due to their inability to metabolize the metals appreciably (Gunther *et al.*, 1999). They have been also used as a bio-accumulation model in assessing the level of chemical pollutants in Columbia (Sherman *et al*., 2009).

Freshwater Asian clam (*Corbicula fluminea*) is one of the important shellfish found in the eastern and central partsof Laguna Lake. These areas are adjacent to the towns of Jala Jala and Binangonan in the province of Rizal. Tons are harvested yearly in these areas and are sold in different towns and nearby provinces including Metro Manila. This is one of the usual food sources of the families residing in the coastal areas of these towns. It is also a source of livelihood of fisher folks in these areas from November to June every year.

Undoubtedly, this shellfish continues to be one of the important products of the lake upon which many depend upon for food and income. Recognizing its importance to the small fishermen and families residing near the coastal areas of the lake, along with the potential problem associated with their bioaccumulation of heavy metals, a study was conducted to assess the levels of heavy metals in freshwater Asian clams in clam habitats in the eastern and center part of Laguna Lake. The risks associated with clam consumption due to the concentrations of arsenic were also taken into consideration in the study.

MATERIALS AND METHODS

Location of the freshwater Asian clam habitats

The clam habitats where the samples were taken are adjacent to Barangay Punta (Study Site 1), Jala Jala, Rizal and Barangay Bangad (Study Site 2), Binangonan, Rizal (Fig. 1). The latter is located in the eastern and the former is in the central bay of Laguna Lake. Freshwater Asian clams are regularly harvested in these areas starting October, continuing up to June, when the water is not too deep.

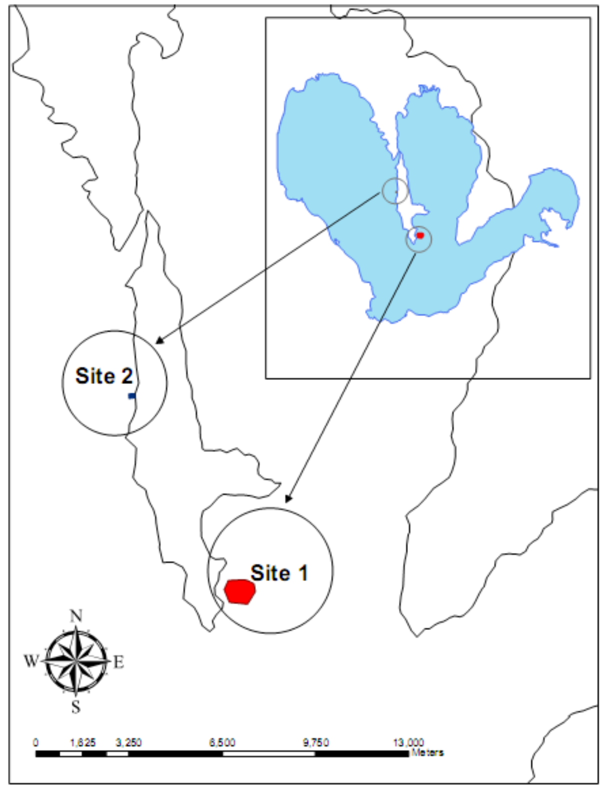

Fig. 1. Locations of the study area

The coordinates of the 1st site are 14.30533 N, 121.249 E; 14.30620 N, 121.25771; 14.30192 N, 121.25370 E and 14.30790 N, 121.25221 (Fig 1). The 2nd site has coordinates of 14.366636 N, 121.21886 E; 14.366735 N, 121.220593 E; 14.365453 N, 121.2207654 E and 14.365231 N, 121.218886 E. Tons of freshwater Asian clams are harvested in these areas yearly and usually sold to nearby markets in Rizal Province and Metro Manila.

Freshwater Asian clam sample collection and preparation

Live freshwater Asian clams were collected in the clam habitats in the summer of 2018 and 2019. A total of 20 kg samples of freshwater Asian clams were randomly collected during each year in the two sites. The samples were thoroughly washed and placed in a clean container with tap water and brought to the Sustainable Agriculture and Natural Resources Institute and the clams were left to cleanse themselves for 8 hours.

Size determination

Twenty clam samples were randomly chosen; each was weighed using a digital balance. The thickness (T), width (W) and length (L, longest portion) of each clam were also measured using a caliper and the clam was classified either as small (<21 mm), medium (21 to 29 mm)or large (>29 mm).

Tissue collection

The remaining clams were classified according to sizes. Soft tissues were shucked from the shells, pooled according to size, thoroughly washed, and placed in a clean container. Four hundred grams of soft tissues were randomly collected from the pooled tissues, placed in a labelled container, stored in a portable freezer and brought to an accredited laboratory for heavy metal analysis.

Heavy metal analysis

The samples were thawed and homogenized prior to acid digestion. The sample solution was initially heated at 60 °C and gradually increased to 120 °C until the nitrous fumes went off. The concentrations of Pb and Cd (in $mg*kg^{-1}$) were determined by Ashing Acid Digestion and Inductively Coupled Plasma Atomic Emission, respectively. The concentration of As was determined through Hydride Vapor Generation and Atomic Absorption Spectrometry. The detection limit for each heavy metals was determined. Appropriate blanks and serial standard solutions were also analyzed using the same procedure to establish the calibration curves. All pooled samples were analyzed in triplicates.

Human health risks assessment

The computation of health risks was based on the methodology developed by the United States Environmental Protection Agency (USEPA, 2004) as well as published research articles. Human health risk computations for As were based on the average life expectancy of Filipinos starting from childhood (5 years of age), then adolescence up to adulthood (64 years old). In order to better assess the risk due to exposure of As, 30% and 100% of inorganic As were considered in the computation of the non-carcinogenic health risk and carcinogenic health risk. The literature suggest that the inorganic form of As is the potent form that can cause health risks to humans, and usually clams contain 30% of it (Munoz *et al.*, 2000; Lorenzana *et al.*, 2009). Other studies however used 100% concentration of As (Sharif *et al.*, 2016; Dela Cruz *et al.*, 2017) in health risks assessments, hence both were considered in the study.

The estimated daily intake (EDI) of As via the consumption of clam tissues was estimated using the equation (Giri and Singh, 2015):

$$EDI = IR \times MC / BW,$$

Where,

IR is the ingestion rate for Asian freshwater clam (75 and 125 g per person per day for the average and heavy consumer, respectively, based on the research of Dela Cruz *et al.* (2017); child consumption is half of adult consumption),

MC is the concentration of As in clam soft tissue ($mg*kg^{-1}$, fresh wt),

BW is the average body weight of Filipinos at varying ages.

The non-carcinogenic health risk of clam consumption due to As exposure, termed as the target hazard quotient (THQ), was computed using the equation (Zhuang *et al.*, 2013):

$$THQ = [(EFr \times ED \times IR \times MC) / (RfDo \times BW \times AT)] \times 10^{-3}]$$

Where:

EFr is exposure frequency (104 and 156 days per year for the average and heavy consumer, respectively, based on the research of Dela Cruz *et al.* (2017);

ED is exposure duration equivalent to the average human lifespan

IR is the ingestion rate (75 or 125 grams per person per day, based on the research of Dela Cruz *et al.*, (2017) for adult and adolescent; child consumption is half of adult consumption);

MC is the concentration of As in clam soft tissue ($mg*kg^{-1}$), fresh weight;

RfDo is the oral reference dose (0.04 $mg*kg^{-1}$) per day based on USEPA (2010) as indicated in Zhuang *et al.*, 2015);

BW is the average body weight of individuals at any age; AT is the averaging time for non-carcinogens (e.g., 10 years or 3,650 days).

The estimate of the potential carcinogenic health effect termed as target Cancer Risk (TR) was based on the incremental probability of an individual to develop cancer over a lifetime exposure to potential carcinogen. This was calculated using the equation (Islam *et al.*, 2015):

$$TR = [(Efr \times ED \times IR \times MC \times CSFo) / (BW \times AT)] \times 10^{-3}]$$

Where:

EFr, ED, IR, MC, and BW assumed the same values in units given for THQ calculations;

CSFo is the oral carcinogenic slope factor equal to 1.5 mg/kg per day, according to the Integrated Risk Information System database of the USEPA as cited by Islam *et al.*, 2015);

AT was adjusted to 365 days per year × number of exposure years (for child, 5; adolescent, 10; and adult 48.6).

RESULTS AND DISCUSSION

Characteristics of freshwater Asian clams in the two study sites

Freshwater Asian clams are typically small, ranging from less than 15 mm across, but sometimes exceeding 30 mm. (McMahon, 2002). Their shell color is normally yellowish to brown or army green.

The size of harvested clams in the clam habitat near Barangay Bangad in Binangonan in 2018 varied from 17 mm to 30 mm in length, with an average of 20 mm and 28.1 mm for small and large, respectively (Table 1). In 2019, small and medium-sized clams with lengths of 18.6 mm and 24.4 mm, respectively, were slightly shorter than those harvested in 2018 with an average lengths of 20 mm and 26.7 mm, respectively. In contrast, clams classified as large in 2019 were 2.3 mm longer compared to those harvested in 2018. The average lengths of small, medium and large clams were 18.6 mm, 24.4 mm and 30.4 mm, respectively.

Clam sizes harvested in the clam habitat adjacent to Barangay Punta, Jala Jala in 2018 varied from 18 mm to 32 mm in length with an average lengths of 23 mm, 27 mm and 30 mm for small, medium and large, respectively (Table 1). In 2019, clam sizes varied from 18 mm to 37 mm, and the average sizes of small, medium and large clams were 18.8 mm, 27 mm and 33.8 mm, respectively.

In 2019, clams harvested in the clam habitat adjacent in Barangay Punta, Jala Jala were slightly longer than those in Barangay Bangad Binangonan. The medium size clams harvested in clam habitat near Barangay Punta, Jala Jala were 2.6 cm longer than those harvested in clam habitat in Barangay Bangad Binangonan. Clams that were classified as large were 3.4 mm longer in the clam habitat near Barangay Punta, Jala Jala than in Barangay Bangad, Binangonan.

The average weight values (Table 2) of the freshwater Asian clam harvested in the clam habitat near Barangay Bangad, Binangonan ranged from 4.32 g to 9.61 g in 2018, whereas the values ranged from 3.89 to 10.39 g in 2019. The small and medium clams were slightly heavier in 2018 than in 2019. In contrast, large clams were 0.78 g heavier in 2019 than in 2018.

Table 2. Average weight (g) of freshwater Asian clam in two study sites

	BINANGONAN		JALA JALA	
Size	2018	2019	2018	2019
Small	4.32	3.89	3.40	2.88
Medium	7.00	6.37	4.80	5.06
Large	9.61	10.39	12.90	14.49

In the clam site adjacent to Barangay Punta, Jala Jala, the average weight of clams harvested varied from 3.40 g to 12.9 g in 2018 and 2.88 g to 14.49 g in 2019. Large clams were heavier in 2019 than in 2018 due to decrease in the frequency of harvesting, which allowed the clam to grow longer and heavier.

Concentrations of heavy metals in freshwater Asian clams in the two study sites

Arsenic and cadmium were detected in clam tissues in both years in the study sites (Table 3). The concentrations of Cd in clams harvested in the clam habitat in Barangay Bangad, Binangonan in both year were 0.05 mg*kg^{-1}, whereas it was 0.04 mg*kg^{-1} in clams harvested in the habitat in Barangay Punta, Jala Jala. It is to be noted that lead concentration in clam tissue was beyond the detection limit of the instrument in all of the study sites.

Table 1. Average size of freshwater Asian clam in two study sites

Size*	BINANGONAN						JALA JALA					
	2018			2019			2018			2019		
	T	W	L	T	W	L	T	W	L	T	W	L
Small	12.2	18.4	**20.0**	10.5	16.5	**18.6**	11.3	19.6	**20.0**	12.0	17.6	**18.8**
Medium	15.0	24.6	**26.7**	12.5	21.1	**24.4**	13.6	24.3	**26.9**	14.5	23.5	**27.0**
Large	16.0	26.1	**28.1**	14.6	25.4	**30.4**	14.7	26.0	**30.0**	18.1	28.0	**33.8**

*Small (<21 mm length), Medium (21-29 mm length), Large (>29 mm length)

Table 3. Concentration of heavy metals in clam tissues in two study sites

Heavy metal ($mg*kg^{-1}$)	BINANGONAN		JALA JALA	
	2018	2019	2018	2019
Arsenic	1.40	1.60	0.90	0.90
Cadmium	0.05	0.05	0.04	0.04
Lead	<0.1	<0.1	<0.1	<0.1

Traces of As were also detected in clam tissues in both sites at a concentration of 1.4 $mg*kg^{-1}$ to 1.6 $mg*kg^{-1}$ in Binangonan and 0.9 $mg*kg^{-1}$ in Jala Jala. Although As has been detected in clams, the concentration is still low compared to the threshold limit set by the International Food Standards (FAO/WHO, 2015) of 2 to 7 $mg*kg^{-1}$.

The presence of heavy metals in clams is an indication of the danger that they may pose to other aquatic species and humans. Biomagnification at the food chain can potentially increase the concentration of these metals at the trophic levels and can create negative effects on humans and aquatic species. Long-term exposure of humans to As in food is mainly related to increased risks of cancer, as well as other skin lesions such as hyperkeratosis and pigmentation changes (Quansah *et al.*, 2015).

Health risks assessment of clam consumption in the study sites

The potential health risks associated with long-term consumption of freshwater Asian clam from childhood to adulthood were estimated. The data used such as life expectancy of Filipinos, average weight of children and adults, were taken from the literature,whereas the concentration of As were based on laboratory analysis of clam tissue samples.

a. Estimated daily intake of As at various stages of human growth

The dietary exposure of consumers to As through the consumption of clam tissues harvested in the clam site in Barangay Bangad, Binangonan from childhood to adulthood was determined using the Estimated Daily Intake (EDI) (Table 4). In 2018, the EDI of clam consumers with moderate clam consumption was 6.95 $mg*kg^{-1}$, 1.0 $mg*kg^{-1}$ lower compared to the computed EDI in 2019.

The EDI values of the moderate and heavy consumers of clams harvested in the clam habitat adjacent to Barangay Bangad, Binangonan (Table 4) were higher in 2019 compared to those in 2018. This is due to the higher concentration of As in the clam tissues, with a difference of 0.20 $mg*kg^{-1}$.

The EDI values (Table 5) of the moderate as well as the heavy clam consumers harvested in the clam habitat near Barangay Punta, Jala Jala, in both year, are the same due to the identical concentrations of As in clam tissues.

Considering the EDI of clams harvested in the two sites and their growth stages, the heavy clam consumers had higher EDI than the moderate clam consumers. This was due to their higher daily ingestion rate. Moreover, teenagers have the highest EDI whereas adults had the lowest EDI due to the difference in average body weight. The average body weight of teenagers was 40.95 kg while adults had an average body weight of 56 kg. All of the estimated EDI values exceeded the WHO/FAO maximum tolerable concentration of 0.30 $mg*kg^{-1}$(Islam *et al.*, 2016), an indication that clam consumption can cause risks especially to continuing consumers.

Table 4. Estimated daily intake ($mg*kg^{-1}*day^{-1}$) of As due to consumption of clam in Brgy Bangad, Binangonan, Rizal

Year	Consumption Level	Child	Adolescent	Adult	Total
2018	Moderate	2.52	2.56	1.87	**6.95**
	Heavy	4.20	4.27	3.12	**11.59**
2019	Moderate	2.88	2.93	2.14	**7.95**
	Heavy	4.80	4.88	3.57	**13.25**

Table 5. Estimated daily intake ($mg*kg^{-1}*day^{-1}$) of As due consumption of clam in Brgy Punta, Jala Jala, Rizal

Year	Consumption Level	Child	Adolescent	Adult	Total
2018	Moderate	1.62	1.65	1.21	4.48
	Heavy	2.70	2.75	2.00	7.45
2019	Moderate	1.62	1.65	1.21	4.48
	Heavy	2.70	2.75	2.00	7.45

b. Target Hazard Quotient (THQ) of As ingestion in the study sites

The THQ is a widely used index developed by USEPA to assess the non-carcinogenic risks of consuming seafood contaminated with As. The estimates were based on 30% and 100% concentration of As. The literature indicated that the concentration of inorganic As in shellfish is about 30%. Other studies, however, used the total As in estimating the THQ of some heavy metals.

Considering the level of As in clam tissues harvested in Barangay Bangad, Binangonan, the highest estimated non-carcinogenic health risks (Table 6) associated in clam consumption at 100% As concentration was 0.122 by the heavy clam consumers in 2019. The THQ of the heavy clam consumers in 2018 was slightly lower compared to the THQ in 2019. Moderate clam consumers had lower THQ for both years compared to the heavy clam consumers due to lower ingestion rate.

The THQ values of heavy clam consumers (Table 7) at 100% and 30% inorganic As concentration in clams harvested adjacent to Barangay Punta, Jala Jala were 0.079 and at 0.033 in both years. The computed THQ values were the same in both years due to the identical concentration of As in clam tissues. Moderate clam consumers had lower THQ, equal to 0.033 compared to the heavy clam consumers due to their lower ingestion rate.

All of the computed THQ in both study sites and years were lower than 1, an indication that exposure to such concentrations of As do not cause any adverse non-carcinogenic health effect due to long-term consumption of clam. The USEPA guidelines prescribe that a THQ higher than 1 signifies that the exposure is higher than the reference dose and it can cause adverse health effects in a lifetime clam consumption by the consumers.

c. Target cancer risk (TR) of arsenic ingestion in the study sites

The estimated lifetime cancer risks (Tables 8 and 9) associated with the consumption of clams with varying concentrations of inorganic As in their bodies in both sites and years fall outside the range of the recommended limit of 1×10^{-6} to 1×10^{-4} set by regulatory agencies abroad (Bogdanovic *et al.*, 2014).

Considering the concentration of As in clams harvested in Barangay Bangad, Binangonan (Table 8), at 30% concentration of inorganic As, only 2 out of 1,000 heavy clam consumers had the lifetime probability of developing a cancer and at 100% inorganic As concentration, 7 out of 1,000 may acquire the same problem at 1.4 mg^*kg^{-1}concentration of As in clam tissues. For moderate clam consumers, 1 out of 1,000 has the lifetime probability of developing cancer at 30% concentration of inorganic As. At 100% concentration, 3 may acquire the same problem.

Considering the 2019 concentration of As in clam tissues, the probability of developing a cancer is slightly higher compared to the 2018 estimated risks. At 30% inorganic As concentration, one additional consumer may develop a cancer and at

Table 6. Estimated THQ of consumers due to consumption of clam with As harvested adjacent in Barangay Bangad, Binangonan, Rizal

Year	Consumption	Child		Adolescent		Adult		Total	
	Level	30%	100%	30%	100%	30%	100%	30%	100%
2018	Moderate	0.004	0.015	0.005	0.018	0.004	0.013	0.013	0.046
	Heavy	0.011	0.039	0.014	0.046	0.010	0.033	0.035	0.118
2019	Moderate	0.004	0.013	0.006	0.021	0.004	0.015	0.014	0.049
	Heavy	0.010	0.032	0.016	0.052	0.011	0.038	0.037	0.122

Table 7. Estimated THQ of consumers due to consumption of clam with As harvested in adjacent sites in Barangay Punta, Jala Jala, Rizal

Year	Consumption	Child		Adolescent		Adult		Total	
	Level	30%	100%	30%	100%	30%	100%	30%	100%
2018	Moderate	0.004	0.012	0.004	0.012	0.003	0.009	0.011	0.033
	Heavy	0.009	0.029	0.009	0.029	0.006	0.021	0.024	0.079
2019	Moderate	0.004	0.012	0.004	0.012	0.003	0.009	0.011	0.033
	Heavy	0.009	0.029	0.009	0.029	0.006	0.021	0.024	0.079

Table 8. Estimated carcinogenic health risks at varying concentrations of As in Barangay Bangad, Binangonan, Rizal

Year	Consumption Level	Child		Adolescent		Adult		Total	
		30%	100%	30%	100%	30%	100%	30%	100%
2018	Moderate	0.0003	0.0011	0.0003	0.0011	0.0002	0.0008	0.0008	0.0030
	Heavy	0.0007	0.0027	0.0008	0.0027	0.0006	0.0020	0.0021	0.0074
2019	Moderate	0.0004	0.0012	0.0004	0.0012	0.0003	0.0009	0.0011	0.0033
	Heavy	0.0009	0.0031	0.0009	0.0031	0.0007	0.0023	0.0025	0.0085

Table 9. Estimated carcinogenic health risks at varying concentrations of As in Barangay Punta, Jala Jala, Rizal

Year	Consumption Level	Child		Adolescent		Adult		Total	
		30%	100%	30%	100%	30%	100%	30%	100%
2018	Moderate	0.0003	0.0007	0.0003	0.0007	0.0002	0.0005	0.0008	0.0019
	Heavy	0.0007	0.0017	0.0008	0.0018	0.0006	0.0013	0.0021	0.0048
2019	Moderate	0.0004	0.0007	0.0004	0.0007	0.0003	0.0005	0.0008	0.0019
	Heavy	0.0009	0.0017	0.0009	0.0018	0.0007	0.0013	0.0021	0.0048

100% concentration, two more consumers have the probability of developing a cancer. The increase is due to the higher concentration of As in the clam tissues.

In Jala Jala (Table 9), at 30% inorganic As concentration in clam, 2 out of 1,000 heavy clam consumers have the lifetime probability of developing cancer and at 100% inorganic As concentration, 5 may acquire the same sickness. For moderate clam consumers, 8 out of 10,000 have the lifetime probability of developing cancer at 30% inorganic As concentration. At 100% concentration, 2 out of 1,000 may acquire the same problem.

Considering the TR estimates in the two study sites, consumption of clam tissues harvested adjacent in Barangay Bangad, Binangonan has a higher probability that a consumer may develop a cancer than of those harvested near Barangay Punta, Jala Jala, Rizal.

CONCLUSION

The following are the conclusions based on the results of the study:

1. The average harvested clams in the clam habitat adjacent to Barangay Punta, Jala Jala, ranging from 18.8 mm to 33.8 mm, were longer compared to those in Binangonan, which ranged from 18.6 mm to 30.4 mm in 2019. Clam sizes were almost the same in the two sites in 2018 except those that were classified as large.
2. Traces of As and Cd were detected in freshwater Asian clam tissues in both years in the two sites. Arsenic had the highest concentration equal to 1.6 mg*kg^{-1} in the clam habitat in Binangonan in 2019, but lower than the National Standards for Arsenic.
3. The concentration of As in freshwater Asian clam in Laguna Lake does not pose appreciable carcinogenic health risks to moderate and heavy clam consumers.
4. The estimated TR's of consumers due to consumption of clam harvested in the clam habitat near Barangay Bangad, Binangonan, Rizal is higher than that in Barangay Punta, Jala Jala, Rizal.
5. The estimated lifetime probability of developing a cancer poses concern. All computed TR's are outside the recommended range, the highest value being obtained in Binangonan in 2019. This implies that clam consumers have the potential of developing a cancer if consumption is high, frequent, and lifetime.

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