Levels of Heavy Metals in Six Aquaculture Commodities Collected from Various Landing Sites of Manila Bay: Relationships with Size and Seasonal Variation

Flordeliza D. Cambia*[1], Kathlene Cleah D. Benitez[1], Karl Bryan S. Perelonia[1], Ulysses M. Montojo[1]

[1]*Fisheries Post Harvest Research and Development Division, National Fisheries Research and Development Institute, Quezon City, Philippines 1103*

ABSTRACT

Fish normally accumulates heavy metals from food, water, and sediments, which can be harmful to human health. In the present study, levels of Pb, Hg and Cd; relationships with size; and seasonal variation in six aquaculture commodities mud crab, *Scylla serrata*; milkfish, *Chanos chanos*; green mussel, *Perna viridis*; oyster, *Crassostrea iridalei*; shrimp, *Penaeus* spp.; and tilapia, *Oreochromis niloticus* from Manila Bay fish landing sites were determined. Homogenized flesh samples were subjected to microwave-assisted digestion in a mixed solution of nitric acid and hydrogen peroxide before analysis by Atomic Absorption Spectrophotometer. All commodities passed the regulatory limit set by Bureau of Fisheries and Aquatic Resources Fisheries Administrative Order 210s 2001 and European Commission 1881/2006 for Pb and Cd. In contrary, 2.04% *O. niloticus* from Hagonoy, Bulacan and Navotas City and 5.00% *Penaeus* spp. from Guagua, Pampanga and Obando, Bulacan exceeded the limit for Hg. Significant negative relationships were found between Cd concentration and fish size in *C. chanos* and *P. viridis*, while significant positive correlations were observed between Pb and fish length in *O. niloticus* and *S. serrata*; Hg and fish length in *P. viridis*; and Cd and fish weight in *Penaeus* spp. Aquaculture commodities were observed to have a significantly higher level of accumulation during the wet season. Results indicate that metal concentration varies among aquaculture commodities, landing sites, and season of catch. Thus, there is a need for risk assessment and regular monitoring for the said commodities.

1. INTRODUCTION

Seafood is an important source of food for hundreds of millions of people around the world because these are high in protein and rich in essential amino acids, micro and macro elements such as calcium, phosphorus, fluorine, and iodine, polyunsaturated fatty acids, and liposoluble vitamins. In 2008, about 81% or equivalent to 115 million tons of estimated world fish production was used as human food with an average per capita of 17 kg (Ismail 2005; Ikem and Egiebor 2005; Food and Agriculture Organization (FAO) 2010).

Despite their nutritive value, consumption of these brings many times a potential hazard concern to human consumers. As these are relatively situated at the top of the aquatic food chain, these can normally accumulate heavy metals from food, water, and sediments (Marsden and Rainbow 2004; Yilmaz and Yilmaz 2007). Essential metals like iron, copper, zinc, and manganese play an important role in biological systems, whereas other metals such as mercury, lead, and cadmium are non-essential as they are toxic even in trace amounts (Türkmen et al. 2008). Heavy metals enter the aquatic environment mainly by anthropogenic sources (Marsden and Rainbow 2004).

In the Philippines, fisheries and aquaculture are among the primary sources of livelihood around the Manila Bay (Partnership in Environmental Management for the Seas of East Asia (PEMSEA) 2001). Aquaculture contributes 41% to the total value of fish production among other fish sectors (Philippine Statistics Authority 2017). Enclosing the bay are aquaculture structures that proliferate mainly along Bataan, Bulacan, and Pampanga in Region III, Cavite in Region IV-A, and in the northern Metro Manila coast-

lines. The most popular culture species are milkfish, tilapia, mudcrab, shrimp/prawn, oyster, and mussel (Manila Bay Area Environmental Atlas 2015).

A previous study was made by Raña et al. (2016) regarding heavy metals (cadmium, Cd; lead, Pb; and mercury, Hg) contamination in six aquaculture commodities from different aquaculture farms around the Bay. The present study focused on heavy metal contamination in Manila Bay landing sites. The objective was to determine the levels of cadmium, lead, and mercury in six important aquaculture commodities: *Scylla serrata*, *Chanos chanos*, *Perna viridis*, *Crassostrea iridalei*, *Penaeus* spp., and *Oreochromis niloticus* in the eleven pre-identified landing sites of Manila Bay. These concentrations were then compared against the recommended maximum levels allowed by the Bureau of Fisheries and Aquatic Resources (BFAR) Fisheries Administrative Order (FAO) 210s 2001 and European Commission (EC)1881/2006. Relationships with size and seasonal variation were also determined.

2. MATERIALS AND METHODS

2.1 Sampling Sites and Collection of Samples

Figure 1 shows the eleven (11) pre-identified landing sites along the Manila Bay area that were sampled for two seasons – March and September 2016 for the dry and wet seasons, respectively. Six different aquaculture commodities were collected specifically mud crab, *S. serrata*; milkfish, *C. chanos*; green mussel, *P. viridis*; oyster *C. iridalei*; shrimp, *Penaeus* spp.; and tilapia, *O. niloticus*. The species being collected per site varied since its collection was dependent on its availability in the landing site.

For the dry season, a total of 498 samples (36 milkfish, 48 tilapia, 14 mud crab, 175 shrimp, 150 green mussels, and 75 oysters) were collected in all landing sites. On the other hand, a total of 510 samples (40 milkfish, 50 tilapia, 20 mud crab, 225 shrimp,

Figure 1. Map of Manila Bay showing the eleven landing sites.

125 green mussels, and 50 oysters) were collected during the wet season.

Samples were freshly bought in the landing sites and were put separately in re-sealable polyethylene bags, correctly labeled and stored in a cooler kept at 0-4°C upon transport to the laboratory for sample preparation and analyses.

2.2 Sample Preparation

Samples were logged and cleaned while the standard length and weight were measured, followed by dissection and homogenization. For the heavy metals analyses, the edible part (i.e., fish muscle) of milkfish, tilapia, and shrimp was used. However, for the analyses of mud crab, oyster, and green mussel, the muscle and viscera were utilized. The homogenized samples were put in re-sealable polyethylene bags with proper labels and stored at approximately -80°C freezer before analyses.

2.3 Sample Analysis

Samples were analyzed for total mercury, lead, and cadmium based on modified Association of Official Analytical Chemists (AOAC) Official Method 999.10 Lead, Cadmium, Zinc, Copper and Iron in Foods – Atomic Absorption Spectrophotometry after Microwave Digestion and modified AOAC Official Method 971.21 Mercury in Food – Flameless Absorption Spectrophotometric Method. For Hg
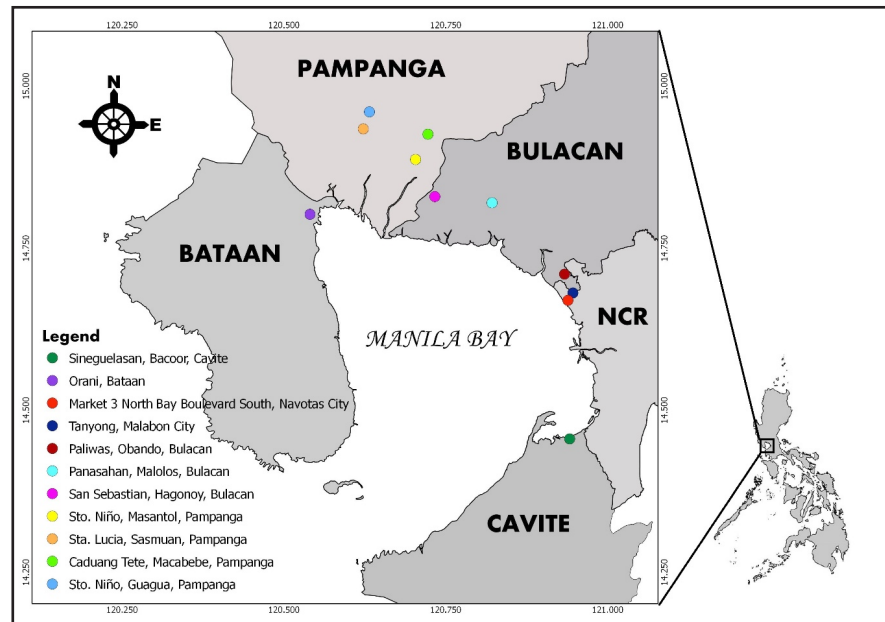
analysis, homogenized samples in triplicates were digested with concentrated nitric acid under pressure in a closed vessel heated by microwave (Ethos One). For Pb and Cd analyses, samples were digested in the same conditions except that the addition of hydrogen peroxide was performed. Digested samples were diluted with 0.1 mol/L nitric acid solution and followed by analytical determinations of heavy metals using the following instruments: (1) Graphite Furnace Atomizer-Atomic Absorption Spectrophotometer (GFA-AAS) for Pb and Cd and (2) Mercury Vaporizing Unit-Atomic Absorption Spectrophotometer (MVU-AAS) for Hg (Horwitz and Latimer 2005).

To guarantee the accuracy and reliability of analytical results, quality assurance and quality control, such as recovery of spiked samples, mid-standards, method blank and reagent blank, were performed during each analysis. Method validation was carried to ensure reliable and accurate results.

2.4 Statistical Analysis

Data analysis was done by means of the statistical software package SPSS 20.0. Levels of heavy metals below the limit of detection (LOD) were not used in the analysis. The Pearson correlation test was used to check for significant relationships between heavy metal concentrations and individual standard length and weight of fishery resources studied. T-test was used to assess seasonal variation. The significance was set at 95% confidence level ($p < 0.05$).

3. RESULTS

Table 1 and 2 show the allowable limit of Pb, Hg, and Cd set by BFAR FAO 210s 2001 and EC

Table 1. Allowable concentration (mg/Kg) of Pb, Hg, and Cd for six aquaculture commodities as set by BFAR (2001) and European Union (2006).

Aquaculture Commodity	BFAR FAO 210s 2001	EC 1881/2006		
	Pb, Hg, Cd	Pb	Hg	Cd
Finfishes (milkfish, tilapia)	0.50	0.30	0.50	0.05
Crustaceans (crab, shrimp)	0.50	0.50	0.50	0.50
Bivalve mollusks (oyster and mussel)	0.50	1.50	0.50	1.00

Table 2. Concentration (mg/Kg) ranges of the six aquaculture commodities from Manila Bay landing sites for the dry and wet season in relation to the allowable limits set by BFAR and European Union.

Aquaculture Commodity	Dry Season			Wet Season		
	Pb	Hg	Cd	Pb	Hg	Cd
C. chanos O. niloticus	<0.30	<0.50	<0.05	<0.30	<0.50 >0.50	<0.05
S. serrata Penaus spp.	<0.50	<0.50	<0.50	<0.50	<0.50 >0.50	<0.50
P. viridis C. iridalei	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50

1881/2006 and the concentration ranges of six aquaculture commodities during the wet and dry season in relation to the allowable limits set by BFAR and European Commission, respectively. All six aquaculture commodities passed the regulatory limit set for Pb and Cd. However, 2.04% of *O. niloticus* collected from Hagonoy, Bulacan and Navotas City; and 5.00% of *Penaeus* spp. samples collected from Guagua, Pampanga and Obando, Bulacan exceeded the allowable mercury concentration of 0.50 mg/Kg.

Table 3 shows the P value of the seasonal comparison of heavy metals per landing site. Analyses showed that for milkfish, only samples collected in Hagonoy, Bulacan had a significant difference in Pb levels between two seasons. For tilapia, only samples collected in Obando, Bulacan had significant difference in Cd levels. Crab samples collected in Orani, Bataan had a significant difference in Cd levels. Shrimp samples obtained from Sasmuan, Pampanga, Obando, Bulacan, and Tanyong, Malabon City had significant differences in Cd concentrations between two seasons. Green mussels from Obando, Bulacan, Tanyong, Malabon City, and Bacoor, Cavite had significant differences in Cd levels between two seasons, and a significant difference in Hg levels in Bacoor,

Table 3. P value of the seasonal comparison of heavy metals per landing site.

Aquaculture Commodity	Landing Sites	Pb	Hg	Cd
C. chanos	Hagonoy, Bulacan			
	P	0.0070	-	-
	Malabon City			
	P	-	-	NS
	Navotas City			
	P	NS	-	-
O. niloticus	Hagonoy, Bulacan			
	P	NS	-	-
	Obando, Bulacan			
	P	NS	-	0.0010
	Malabon City			
	P	-	-	NS
S. serrata	Orani, Bataan			
	P	-	-	<0.0010
	Hagonoy, Bulacan			
	P	-	-	NS
Penaeus spp.	Macabebe, Pampanga			
	P	-	-	NS
	Sasmuan, Pampanga			
	P	NS	-	0.0010
	Obando, Bulacan			
	P	NS	-	0.0090
	Malabon City			
	P	-	NS	0.0040
P. viridis	Orani, Bataan			
	P	-	-	NS
	Obando, Bulacan			
	P	-	-	0.0190
	Malabon City			
	P	-	-	0.0001
	Bacoor, Cavite			
	P	-	0.0280	<0.0010
C. iridalei	Malolos, Bulacan			
	P	-	-	NS

P   Probability value, ($p<0.05$)
NS   Not significant
-     No seasonal comparison made due to ND values

Cavite. There was no significant difference found on heavy metal concentrations in oyster between two seasons. Landing sites which were not included in Table 3 were observed to have heavy metal concentrations below the LOD.

The length and weight of each species were correlated to its heavy metals concentrations using Pearson Correlation as presented in Table 4. Results of the said analysis showed that, except in a few cases, relationships between metal concentrations and fish size were negative.

Between the size and Pb concentration measured for each species, negative correlations were found in milkfish, shrimp, green mussel, and oyster. Positive correlations between weight and Pb were found in both tilapia and mud crab. Significant positive correlations related to length were recorded in tilapia and mud crab.

Significant negative relationships were observed between length and Cd concentrations of milkfish and green mussel. The same relationship existed between weight and Cd concentrations in green mussel and oyster. A significant positive relationship was observed between shrimp weight and Cd. Negative associations of Cd concentration related to the fish weight were found in milkfish and tilapia and the same association existed between length of tilapia and Cd. Positive correlations between Cd and length were detected in mud crab and shrimp and the same correlation was present between mud crab weight and Cd.

Between the fish size and Hg concentration measured for each species, significant positive correlations were found in green mussel. Positive correlations were found between shrimp size and Hg. The same correlation was detected between tilapia weight and Hg. While negative correlations were found in mud crab both in length and weight; and the same correlation between tilapia length and Hg. There was no correlation made with milkfish and oyster since Hg concentrations were below the LOD.

4. DISCUSSION

All aquaculture commodities collected from various landing sites passed the regulatory limit set by BFAR FAO 210s 2001 and EC 1881/2006 for lead and cadmium. In contrary, two of 98 (2.04%) *O. niloticus* from Hagonoy, Bulacan and Navotas City and four of 80 (5.00%) *Penaeus* spp. samples from Guagua, Pampanga and Obando, Bulacan exceeded the 0.50 µg/g regulatory limit set for mercury. The present study recorded a lower percentage of heavy metals contamination when compared with the study of Raña et al. (2016) on similar aquaculture commodities collected from culture ponds.

As habitat influenced heavy metal concentrations in the different aquaculture commodities, the present study showed that most of the commodities which recorded the highest concentration were found in landing sites located at Bulacan. According to the annual report by Blacksmith Institute (2006), contamination of the Marilao-Meycauayan-Obando River system comes from both formal and informal industries, such as used lead-acid battery recycling, tanneries, gold and precious metals refining, jewelry making, lead smelters, and numerous open dumpsites. Thus, the effluents, emissions, and wastes produced find their way into the atmosphere, river system, and eventually to fishponds before finally exiting the Manila Bay.

Season of catch could also lead to different metal concentrations. In the present study, significantly higher levels of most of the heavy metals were observed during the wet season. The previous study made by Raña et al. (2016) also showed a higher concentration of Pb during the wet season. Urban stormwater discharge during wet-weather flows is a major contributor to the pollution of many receiving water. Agricultural runoff is also one of the nonpoint sources of pollution that also affects water quality (Lee et al. 2004; Nordeidet al. 2004). As the surrounding inland area of Manila Bay area is predominantly agriculture and many manufacturing industries are also found in industrial parks both in coastal and non-coastal areas of the Bay, urban and agricultural runoffs can significantly affect the quality of the surrounding water bodies and biota (Check 1997; Mason et al. 1999; Turer et al. 2001; Duzgoren-Aydin et al. 2004; PEMSEA 2001).

Heavy metal concentrations and fish sizes were correlated and observed to have negative relationships in most cases than positive relationships. Significant positive correlations were mostly found between fish size and Hg and Pb concentrations, while significant negative correlations were mostly found Cd concentrations. According to a previous study, the concentration of Hg and Cd in emperor fish *Lethrinus lentjan* demonstrated a positive correlation with fish length and weight (Al-yousuf et al. 2000). An assessment of heavy metal contamination of marine biota (fish and various bivalves) was made in the Gulf of Oman, where total Hg concentrations were found to generally increase with the age and size of the fish (de

Table 4. Pearson correlation coefficient (R) and probability value (P) for the relationships between heavy metal concentrations and fish size (weight, g and length, cm)

Aquaculture Commodity	Size Ranges	Data	Pb	Hg	Cd
C. chanos					
Weight	109.32-915.52	R	-0.0074	-	-0.3021
		P	NS	-	NS
Length	19.00-36.50	R	-0.0590	-	-0.4293
		P	NS	-	0.0363
O.niloticus					
Weight	104.53-487.36	R	0.2927	0.3540	-0.1709
		P	NS	NS	NS
Length	13.70-27.30	R	0.3623	-0.1440	-0.2578
		P	0.0352	NS	NS
S. serrata					
Weight	118.78-850.25	R	0.5962	-0.4685	0.2764
		P	NS	NS	NS
Length	9.00-17.00	R	0.8067	-0.1787	0.3272
		P	0.0027	NS	NS
Penaeus spp.					
Weight	12.19-37.63	R	-0.3251	0.2958	0.3066
		P	NS	NS	0.0361
Length	12.08-17.94	R	-0.2844	0.2997	0.1531
		P	NS	NS	NS
P. viridis					
Weight	4.56-24.82	R	-0.1221	0.7996	-0.3305
		P	NS	0.0018	0.0218
Length	4.30-8.06	R	-0.4697	0.7650	-0.4944
		P	NS	0.0037	0.0004
C. iridalei					
Weight	19.35-86.72	R	-0.4943	-	-0.8591
		P	NS	-	<0.0010

NS   Not significant
-    No correlation made due to ND values

Mora et al. 2004). Guo (2005) found that for lancelet *Branchiostoma belcheri*, a positive correlation existed between concentrations of Hg and Cd with fish length and weight, nevertheless, no significant relationship was found between Pb and fish length and weight. Widianarko et al. (2000) found out that there was a significant decline in lead concentrations with the increase in the size of guppy *Poecilia reticulata*. Some research has shown negative relationships between fish size and the metal concentrations found in those fishes (Canli and Atli 2003; Farkas et al. 2003; Yi and Zhang 2012).

Trace element accumulation in living organisms which is controlled by ecological needs, swimming behaviors, specific uptake, detoxification, and elimination mechanisms, depends significantly on the metabolic rate of organisms (Newman and Doubet 1989; Al-majed and Preston 2000; Jung and Zauke 2008; Kasimoglu 2014). Some metals do not increase in concentrations with age or size because they are thought to be under homeostatic control (Evans et al. 1993; Kasimoglu 2014). Thus, in the present study, the positive correlation between some metals and fish sizes may be due to loss of homeostasis capacity of the species under chronic metal exposure leading to bioaccumulation. This assumption is supported well also by the fact that lipid as a percent of body weight is usually lower in younger fish, decreases during spawning and reaches its peak at the end of the main feeding period (Weatherrly and Gill 1987; Kasimoglu 2014).

5. CONCLUSION

The results obtained from this study indicate that heavy metal concentration varies among aquaculture commodities, landing sites, and season of catch. Size-specific metabolic rates connected with growth may contribute significantly to this variation. Runoffs which have little or no treatment may also contribute to the higher accumulation of heavy metals during the wet season. From the standpoint of food safety, there is a need for risk assessment and regular monitoring for the said aquaculture commodities.

6. ACKNOWLEDGMENT

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