Heavy Metal Contamination in Water and Fishery Resources in Manila Bay Aquaculture Farms

Perelonia, Karl Bryan S., Abendanio, Camille C., Raña, Joan A.*, Opinion, April Grace R., Villeza, Jordan T., and Cambia, Flordeliza D.

Fisheries Post Harvest Research and Development Division,
National Fisheries Research and Development Institute, Quezon City, Philippines 1103

Abstract

Heavy metals (HM) are high-atomic weight elements hazardous at very low concentrations. Despite the health risk HM contamination brings, studies conducted were only confined to the offshore and marine portions of the bay. Hence, this study was conducted to establish baseline information and compare the spatial and seasonal distribution of heavy metal contamination in water and fishery resources in aquaculture farms and coastal areas in Manila Bay. Water and major aquaculture commodities were collected in November 2014, February 2015 and April 2015 and were analyzed for lead (Pb), cadmium (Cd), and mercury (Hg) using the following methods: (1) Graphite Furnace Atomizer-Atomic Absorption Spectrophotometry (GFA-AAS) for Pb and Cd and (2) Mercury Vaporizing Unit-AAS (MVU-AAS) for Hg. Cd (Bataan, Cavite, Bulacan, and Pampanga) and Hg (Cavite and Pampanga) in water were found significantly higher during the dry season ($p<0.05$). In contrast, Pb in water was relatively higher during the wet season but levels were not significantly different with those in the dry. Several sites in Bataan, Bulacan, and Cavite, exceeded DENR regulatory limits for Pb and Hg in water by 1.35 to 1.8%. As for the fishery commodities, 2/12 milkfish samples and 1/9 exceeded regulatory limit for Pb in finfish (0.3 mg/kg) by 1.03 to 3.57% while 1/12 milkfish samples and 1/13 oyster samples exceeded the limit for Hg in bivalves (0.5 mg/kg) by 0.45 to 0.75%.

Keywords: heavy metals, aquaculture, Manila Bay

Introduction

Heavy metals (HM) are high-atomic weight elements hazardous at very low concentrations. These primarily include cadmium (Cd), lead (Pb), and mercury (Hg) (Tiimub & DzifaAfua, 2013). Heavy metals can enter the aquatic ecosystem from different natural and anthropogenic sources such as domestic wastewater, pesticides and inorganic fertilizers, geologic weathering as well as shipping and harbor activities (Krishna *et al*, 2014). Heavy metal pollution creates an immense threat owing to their persistence in the aquatic environment and accumulation in the organisms. Their entering the food chain increases public health risks (Su *et al*, 2009).

Fish absorbs metals through ingestion of contaminated water, sediments, suspended solids, and prey organisms. Heavy metals have also been observed to undergo bioaccumulation and bio-magnification in the tissue of aquatic organisms (Ebrahimi and Taherianfard, 2011). The rate of bioaccumulation of HM in aquatic organisms depends on several factors like fish species, ability to digest the metals, feeding habits, age of fish, lipid content in the tissue, and the concentration of such metal in the area (Eneji, 2011; Khayatzadeh and Abbasi, 2010; Su *et al*, 2009). Heavy metals accumulate mainly in kidneys, adrenal glands, liver, lungs or gills in fish, hair and skin (Martin & Griswold, 2009).

Heavy metals, in trace amounts, have different roles for aquatic organisms' proper physiological functions (Khayatzadeh and Abbasi, 2010). However, water bodies with a high amount of these negatively affect the aquatic organisms. Heavy metal pollution primarily inhibits the growth of fish and other aquatic organisms. It also reduces the survival of fish larvae, which may considerably reduce the fish population or result in their extinction.

Furthermore, contaminated sediments may cause death in benthic organisms which implies reduced food availability for larger animals such as fish. Behavioural anomalies may also develop such as impaired locomotors performance resulting in increased susceptibility to predators or structural damages, mainly vertebral deformities. In humans, exposure to copper, for example, inhibit skeletal ossification, while lead cause scoliosis (Khayatzadeh and Abbasi, 2010; Sharma *et al*, 2014).

Human consumption of contaminated fish and other aquatic organisms may result in transfer and accumulation of metals in the human body posing a threat on consumer health and safety. Accumulation of high amounts of heavy metals may cause high blood pressure, cancerous changes, and damage to kidneys, liver, and brain. In some cases they may also lead to mental disorders and loss of brain function (Martin & Griswold, 2009).

Accumulation of cadmium, for example, affects the liver, kidney, lung, bones, placenta, brain, and the central nervous system. Other damages such as reproductive and development toxicity, hepatic, haematological, and immunological effects in character may also result (Morais *et al*, 2012). Moreover, chronic lead intoxication in adults can cause anaemia, cancer, reproductive harm in males, and hormonal imbalance of vitamin D metabolism (WHO, 1995). Exposure to high levels of metallic, inorganic, or organic mercury can permanently damage the brain, kidneys, and even the developing fetus (Morais *et al*, 2012).

Manila Bay is known as one of the premier fishing grounds in the country making fishing as the primary source of livelihood around the bay. Surrounding coastal communities are benefited by the bountiful aquatic resources. Moreover, the once varied ecological habitats like sea grasses, corals, and mangroves in the bay area serve as the breeding grounds of fish life and also serve indirect ecological functions (e.g. shoreline stabilization, storm barriers, and carbon storage) (PEMSEA, 2006).

Aquaculture in the provinces around Manila Bay was first documented in 1932 with the introduction of oyster farming in Binakayan, Cavite (Yap, 1999), one of the most productive aquaculture areas in the country. Manila Bay accounts for an average of almost 50% of the national production of tiger prawn and oyster (PEMSEA, 2006). As reported by BAS (2012), the volume of aquaculture production in Manila bay provinces (Bataan, Pampanga, Bulacan, Cavite, and NCR) in 2010 has reached 335,985.80 MT, 3% higher than 2009 production of 314,110.74 MT. However, all these resources, which are quite beneficial to all Filipinos especially those living near the bay, are constantly under threat. Population expansion, rapid urbanization, uncontrolled coast, and basin development and mismanagement of resources are apparent in many areas around the bay. Products of modernization are considered major threats to the bay's sustainability and productivity because these products result in water pollution, including HM pollution.

Several studies have been conducted to assess the contamination of HM in Manila bay. According to Su *et al* (2009), HM particularly total chromium, total lead and total cadmium were evident in the bay waters, fish and macroinvertebrates. The study of Velazquez *et al* (2010) showed that the total dissolved copper and cadmium were labile while total dissolved zinc was organically bound. Elevated levels of these metals near point sources suggest anthropogenic inputs in the bay. Results of the risk assessment conducted by PEMSEA and MBEMP TWG-RRA (2004) showed that the concentrations of HM in water around river mouths were higher than in the water inside the bay, suggesting that the contribution of land-based human activities is a major source of pollution. Similar results were observed in the case of HM in the sediments. In the case of HM concentration in the organisms' tissues, scad (0.067 mg/kg), sardines species (1.39 mg/kg), and crevalle (0.296 mg/kg) are among the pelagic fish that appear to have a high bioaccumulation of metals. HM in shellfish (mussels and oysters) from Manila Bay, specifically from the mouth of the Pasig River, may pose a relatively significant risk to human health since concentrations showed high RQ values of 3.8 to 7 for lead (Pb).

These studies were confined to the offshore and marine portions of the bay only. It was only lately that Dr. Marlo Mendoza (unpublished) obtained data concerning HM concentrations in freshwater and brackishwater aquaculture species in areas surrounding Manila Bay. Results of monitoring conducted from January to December 2008 showed that heavy metals, such as As, Cd, Cu, Mn, Ni, Zn, Pb, Cr, and Hg were present in milkfish, tilapia, prawns, green mussel, clam "*paros*", and oysters collected from the area. The levels ranged from non-detectable (ND) to several folds higher than the standard limits set by BFAR (Fisheries Administrative Order 210 Series of 2001) and US Environmental Protection Agency, EPA (2000). Based on standards set by Codex Alimentarius Commission (CAC 206), European Union EC1881-206 and US FDA Centre for Food Safety and Applied Nutrition (CFSAN), however, these are acceptable. These results prompted BFAR to conduct a verification study, which also aims to generate baseline information on the possible contamination of heavy metals in fish and fishery products from Manila bay and adjacent aquaculture areas.

This study aims to establish baseline information and compare the spatial and seasonal distribution of heavy metal contamination in water and fishery resources in aquaculture farms and coastal areas in Manila Bay.

Methodology

Sampling Sites and Collection of Samples

Blocking strategy and sampling sites used in Chapter 2 was employed in this study. Forty-seven pre-identified aquaculture farms were sampled each season –November 2014 for the wet season and February and April 2015 for the dry season. River tributaries of the aquaculture farms were also sampled for comparison.

WATER SAMPLES

Water samples about one liter (1L) were collected in composite for heavy metal analyses and were placed in appropriate container, preserved with acid, labeled and transported to the laboratory in a temperature-controlled cooler. Storage and preservation of collected water samples were done according to US-Environmental Protection Agency (EPA), Sampling and Analysis of Waters, wastewaters soils and Wastes (2009).

Eighty one (81) water samples (35 pond, 35 water source, 2 pen, and 9 coastal area) were collected during the wet season (November 2014), while 82 water samples (35 pond, 35 water source, 3 pen, and 9 coastal area) were collected during the dry season (February and April 2015).

FISH SAMPLES

Six kinds of farmed fishery resources were collected from the aquaculture farms for the study – tilapia, milkfish, shrimp, crab, mussel, and oyster. However, sample species and number of samples collected per site were variable, as sample collection is solely dependent on the availability of samples.

Fifty six (56) samples of aquaculture commodities (12 milkfish, 9 tilapia, 7 crab, 5 shrimp, 13 oyster, and 10 mussel) were collected throughout the sampling period. Freshly harvested fish and shellfish of at least one kilogram (1kg) per species were put separately in an appropriately-labeled re-sealable polyethylene bags and placed in a temperature-controlled cooler kept at 0 to 4°C for transport at laboratory for analyses.

Fish samples were cleaned and dissected, composited and homogenized to make up at least 200 grams. Homogenized samples were stored in re-sealable polyethylene bags and placed in the ultralow freezer (-80°C) prior to analysis.

Method of Heavy Metal Analyses

Water samples were analyzed for total mercury (Hg), lead (Pb), cadmium (Cd) and hexavalent chromium (Cr^{+6}). As for the fish and fishery resources, all the above-mentioned HM analyses were conducted except for the hexavalent chromium. The method used for both water and fish was the closed vessel-microwave-assisted-acid digestion adopting the method of the manufacturer, followed by analytical determinations of metals using the following instruments: (1) Graphite Furnace Atomizer-Atomic Absorption Spectrophotometer (GFA-AAS) for Pb and Cd and (2) Mercury Vaporizing Unit AAS (MVU-AAS) for Hg. The reference methods used for the analysis of heavy metals in water were US-EPA 3015a, 2007(Cd, Hg, and Pb) and US-EPA 7196a 1992, (hexavalent chromium) and for fish samples, modified AOAC 2013.06 was used.

To ensure the accuracy and reliability of analytical results, the laboratory performed quality assurance and quality control schemes (e.g. mid-standards, recovery of fortified samples, and method/reagent blanks) and other performance characteristics for method validation (e.g. repeatability, reproducibility and accuracy using Certified Reference Materials).

Statistical Analyses

The levels of HM in the aquaculture farms and water sources during the wet and dry seasons were compared using t-test, as well as its seasonal variations. On the other hand, HM in the fishery commodities collected during the wet and dry seasons were compared using Analysis of Variance (ANOVA). All statistical analyses were carried out using SPSS version 20.

Results

Levels of Heavy Metals in Water

Levels of heavy metals in the aquaculture farms varied among the different blocks. The following are the ranges of the levels listed from the most abundant to the least: lead (ND to 0.0759 mg/L); cadmium (ND - 0.0028 mg/L); mercury (ND to 0.0065 mg/L); and hexavalent chromium (ND).

LEAD

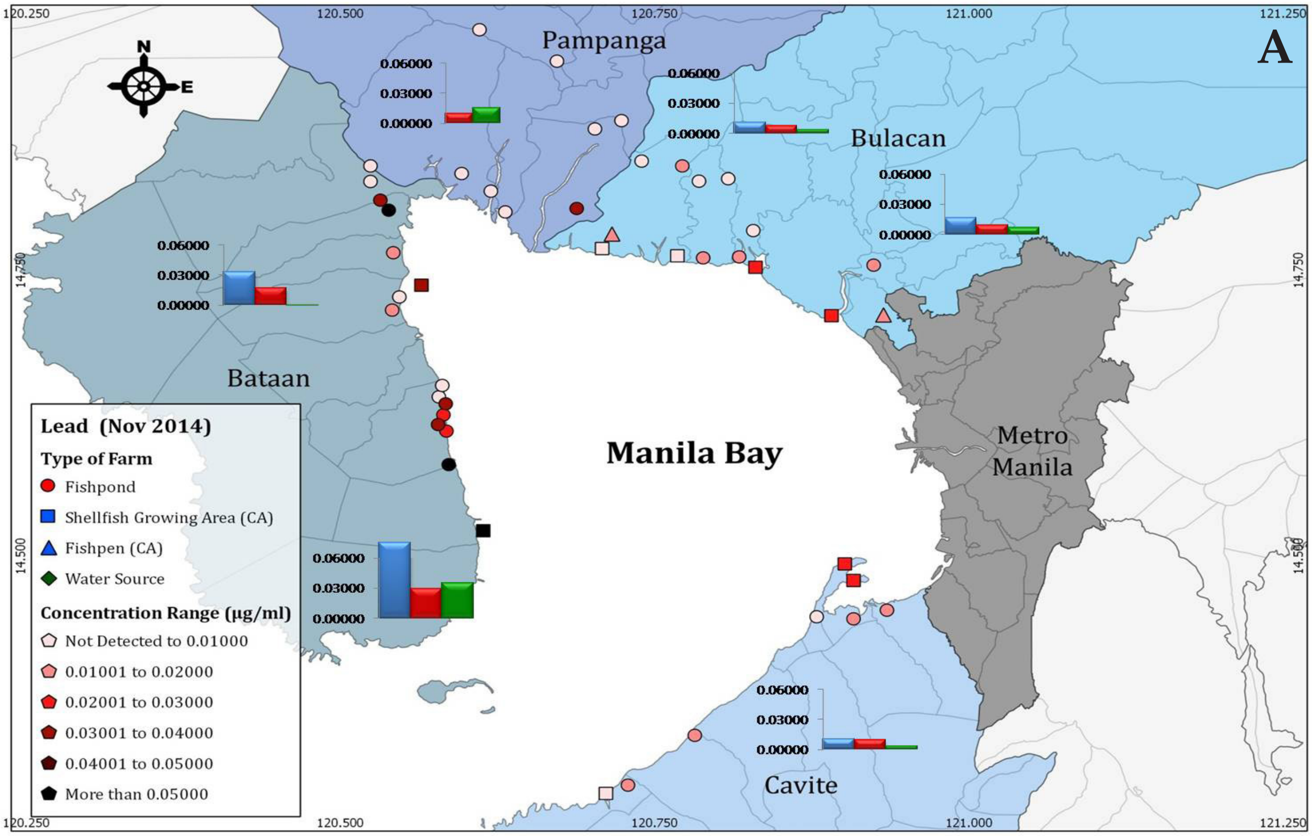
Table 4.1 summarizes the values of lead concentration obtained for the both sampling periods, while its spatial distribution in aquaculture farms are presented in Figure 4.1.

Lead levels in the aquaculture farms for the both sampling season were found to be variable, with values ranging from ND to 0.0759 mg/L. The highest concentration was observed in a pond water sample from Southern Bataan (November 2014).

During wet season, levels of lead ranged from ND to 0.0759 mg/L, with three sampling sites (one in Northern Bataan and two in Southern Bataan) exceeding DENR regulatory limit of 0.05 mg/L, and were found relatively higher compared to the dry season, with levels of Lead ranging from ND to 0.0251 mg/L and all sites conforming with the limit. However, there was no significant difference in the levels of lead in the different blocks in both sampling periods ($p > 0.05$, refer to Table 4.2). In addition, statistical analysis showed that lead concentration in aquaculture farm (pond, pen and coastal waters) and their respective water sources had no significant difference ($p>0.05$), as shown

Table 4.1. Lead concentrations in aquaculture farms for wet (November 2014) and dry season (February and April 2015.

BLOCK	Mean Lead Concentration (ppm, mg/L)	Lead Concentration Range (ppm, mg/L)
Block 1 (Eastern Bulacan)	0.0143	0.0022 – 0.0251
Block 2 (Western Bulacan)	0.0085	0.0014 – 0.0185
Block 3 (Northern Bataan)	0.0129	ND – 0.0502
Block 4 (Southern Bataan)	0.0232	0.0035 – 0.0759
Block 5 (Cavite)	0.0089	ND – 0.0178
Block 7 (Pampanga)	0.0078	ND – 0.0308



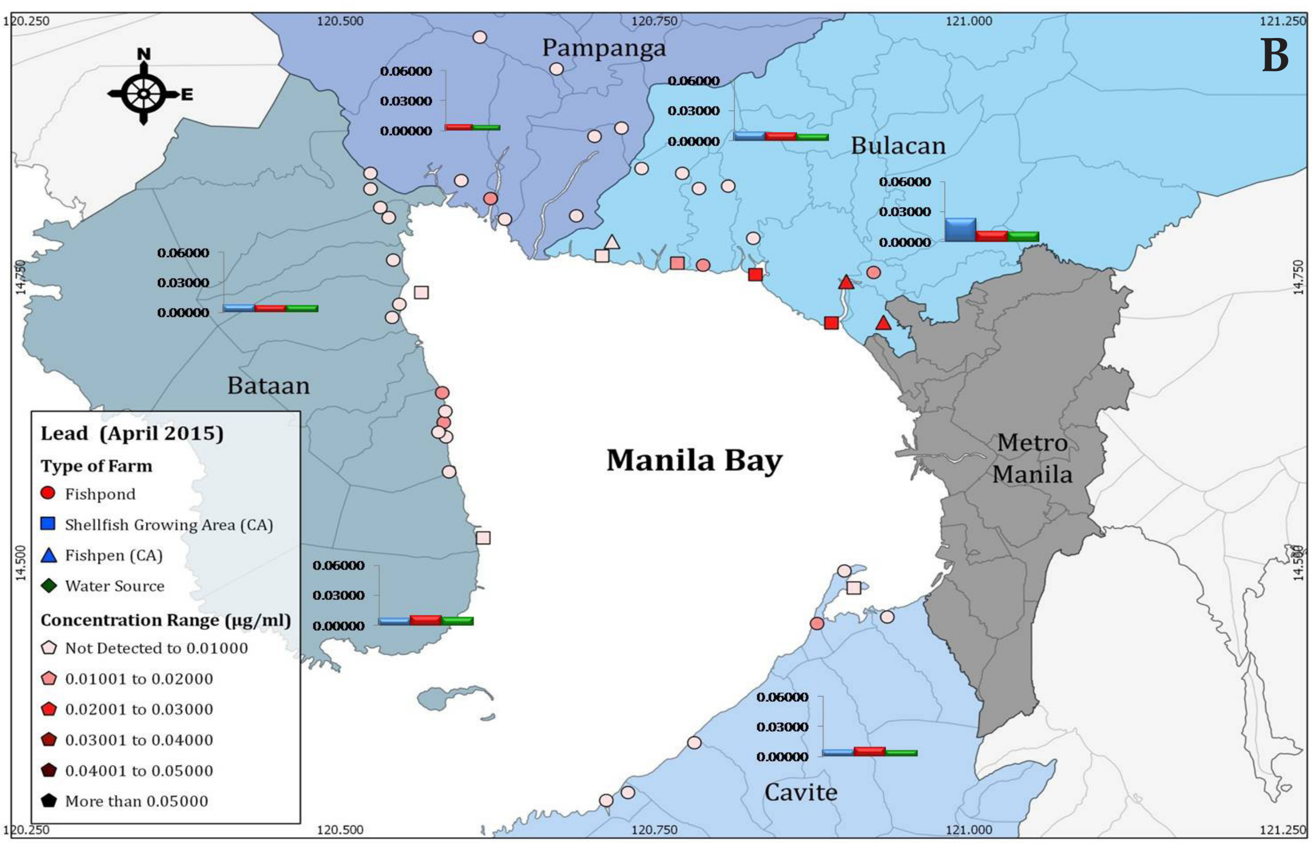


Figure 4.1. Levels of lead in coastal areas/fish pens, ponds, and water sourcesin November 2014 (A) and April 2015 (B).

Table 4.2. Seasonal comparison of heavy metal concentration in aquaculture farms.

Sampling Sites	Cd, mg/L		Hg, mg/L		Pb, mg/L	
	Wet	Dry	Wet	Dry	Wet	Dry
B1	0.00016	0.00020	0.00010	0.00100	0.0092	0.00844
	P = 0.681		P = 0.298		P = 0.863	
Significance, $\alpha_{0.05}$	Not Significant		Not Significant		Not Significant	
B2	0.00016	0.00076	0.0007	0.0007	0.0075	0.0077
	P = 0.098		P = 0.172		P = 0.927	
Significance, $\alpha_{0.05}$	Not Significant		Not Significant		Not Significant	
B3	0.00007	0.00027	0.0004	0.0006	0.01746	0.0061
	P = 0.004		P = 0.529		P = 0.249	
Significance, $\alpha_{0.05}$	Significant		Not Significant		Not Significant	
B4	0.00042	0.00088	0.0012	0.0005	0.02953	0.0097
	P = 0.009		P = 0.342		P = 0.069	
Significance, $\alpha_{0.05}$	Significant		Not Significant		Not Significant	
B5	0.0002	0.0012	0.00004	0.00374	0.0098	0.0088
	P = 0.012		P = 0.000		P = 0.773	
Significance, $\alpha_{0.05}$	Significant		Significant		Not Significant	
B7	0.0003	1.4934	0.0001	0.0012	0.0096	0.006
	P = 0.016		P = 0.000		P = 0.394	
Significance, $\alpha_{0.05}$	Significant		Significant		Not Significant	

in Table 4.3. This indicates that neither the aquaculture farm nor the water source affected the level of lead in the other.

CADMIUM

Reflected in Figure 4.2 are the levels of cadmium in the different sampling sites during the wet and the dry seasons. On the other hand, Table 4.4 shows the cadmium concentrations obtained throughout the sampling period.

Cadmium levels during the dry season (range of ND - 0.0028 mg/L) were significantly higher ($p<0.05$) compared to the wet season were comparable with all the blocks. All sam-(range of ND - 0.0023 mg/L). Cadmium concentrations in Cavite and Pampanga were significantly higher ($p<0.01$) than in Western Bulacan, Eastern Bulacan, and Northern Bataan. Those in Southern Bataan, on the other hand, pling sites passed the DENR regulatory limit of 0.01 mg/L for cadmium in water.

T-test results showed that cadmium levels in the aquaculture farms in Northern Bataan with an average cadmium concentration of 0.00017 mg/L, were significantly higher ($p<0.05$) than the water source (mean = 0.00015 µg/ml)during wet season, as indicated in Table 4.3.

MERCURY

Levels of mercury in the aquaculture farms during the wet and dry seasons are presented in Figure 4.3. On the other hand, Table 4.5 shows the mercury concentrations obtained throughout the sampling period.

Table 4.3. Comparison of concentrations of heavy metal in pond collected from different sites of Manila Bay during wet and dry seasons.

WET

Sampling Sites	Cd, mg/L		Hg, mg/L		Pb, mg/L	
	Pond	River	Pond	River	Pond	River
B1	0.0002	0.0002	0.0012	0.0011	0.0101	0.0096
	P = 0.802		P = 0.215		P = 0.652	
Significance, $\alpha_{0.05}$	Not Significant		Not Significant		Not Significant	
B2	0.0008	0.0011	N.D.	N.D.	0.0077	0.0033
	P = 0.0574		No variance		P = 0.151	
Significance, $\alpha_{0.05}$	Not Significant				Not Significant	
B3	0.0001	0.00001	0.0003	0.0002	0.0175	0.0004
	P = 0.018		P = 0.449		P = 0.044	
Significance, $\alpha_{0.05}$	Significant		Not Significant		Significant	
B4	0.0004	0.0003	0.0012	0.0002	0.0295	0.0352
	P = 0.578		P = 0.142		P = 0.739	
Significance, $\alpha_{0.05}$	Not Significant		Not Significant		Not Significant	
B5	0.0002	0.0002	0.00004	0.00012	0.0098	0.0036
	P = 0.423		P = 0.545		P = 0.125	
Significance, $\alpha_{0.05}$	Not Significant		Not Significant		Not Significant	
B7	0.0003	0.0009	0.0001	0.00006	0.0096	0.00155
	P = 0.105		P = 0.337		P = 0.224	
Significance, $\alpha_{0.05}$	Not Significant		Not Significant		Not Significant	

DRY

Sampling Sites	Cd, mg/L		Hg, mg/L		Pb, mg/L	
	Pond	River	Pond	River	Pond	River
B1	0.0002	0.0002	0.0012	0.0011	0.0101	0.0096
	P = 0.539		P = 0.619		P = 0.915	
Significance, $\alpha_{0.05}$	Not Significant		Not Significant		Not Significant	
B2	0.0002	0.00005	0.0007	0.0002	0.0075	0.0064
	P = 0.487		P = 0.667		P = 0.579	
Significance, $\alpha_{0.05}$	Not Significant		Not Significant		Not Significant	
B3	0.0003	0.0003	0.0006	0.0004	0.0064	0.0064
	P = 0.562		P = 0.200		P = 0.835	
Significance, $\alpha_{0.05}$	Not Significant		Not Significant		Not Significant	
B4	0.0009	0.0009	0.0005	0.00003	0.0098	0.0079
	P = 0.906		P = 0.378		P = 0.388	
Significance, $\alpha_{0.05}$	Not Significant		Not Significant		Not Significant	
B5	0.0012	0.0011	0.0037	0.0031	0.0088	0.0046
	P = 0.857		P = 0.164		P = 0.341	
Significance, $\alpha_{0.05}$	Not Significant		Not Significant		Not Significant	
B7	0.0015	0.0012	0.0012	0.00043	0.006	0.0051
	P = 0.643		P = 0.006		P = 0.565	
Significance, $\alpha_{0.05}$	Not Significant		Significant		Not Significant	

Table 4.4. Cadmium concentrations in aquaculture farms for wet (November 2014) and dry season (February and April 2015.

BLOCK	Mean Cadmium Concentration (ppm, mg/L)	Cadmium Concentration Range (ppm, mg/L)
Block 1 (Eastern Bulacan)	0.0003	0.00004 – 0.00050
Block 2 (Western Bulacan)	0.0007	ND – 0.0027
Block 3 (Northern Bataan)	0.0002	ND – 0.0004
Block 4 (Southern Bataan)	0.0006	0.0002 – 0.0011
Block 5 (Cavite)	0.0008	0.0001 – 0.0019
Block 7 (Pampanga)	0.0009	ND – 0.0028

Mercury levels during the dry season (range of ND - 0.0065 mg/L) were higher than in the wet season (range of ND - 0.0037 mg/L). Significantly higher mercury levels during the dry season were observed in Cavite and Pampanga ($p<0.05$).

Three out of 46 sampling sites, all noted in Southern Bataan during the wet season exceeded DENR regulatory limit of 0.002 mg/L for mercury in water. For the dry season, 14 out of 47 sites – three from Eastern Bulacan, one in Western Bulacan, Northern Bataan, and Southern Bataan, and eight in Cavite – failed to meet the DENR regulatory limit.

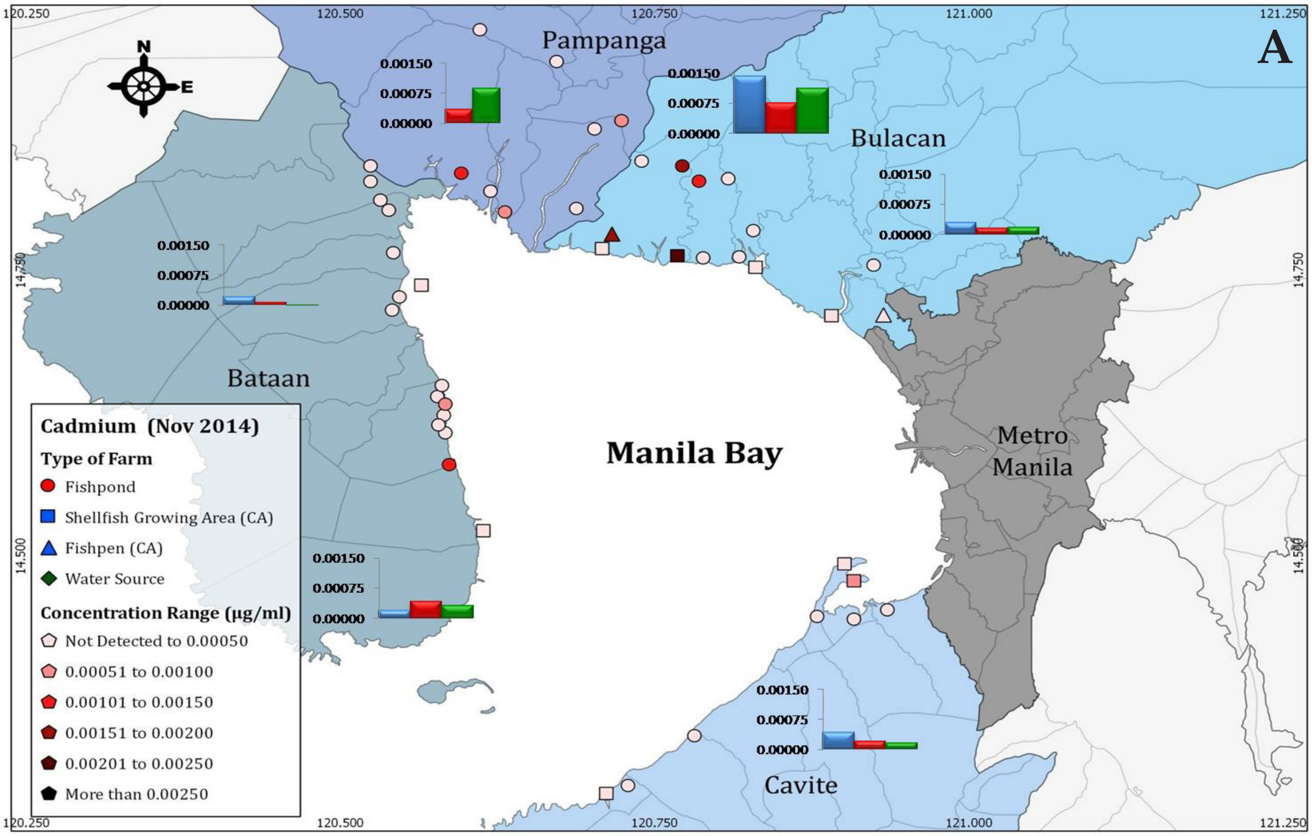
T-test results showed that only Pampanga had significantly higher ($p<0.05$) mercury levels in the aquafarm than its water source, as indicated in Table 4.3.

Levels of Heavy Metals in Fish and Fishery Resources

Heavy metals in fish and fishery resources had no significant difference ($p>0.05$) during the wet (November) and dry (February and April) seasons except for lead in crab (refer to Table 4.6). The following are the ranges and mean values of heavy metals in aquaculture commodities collected arranged in descending order: lead (ND - 1.0723 mg/kg; 0.1463 mg/kg); cadmium (ND - 0.5526 mg/kg; 0.0606 mg/kg); and mercury (ND - 1.1063 mg/kg; 0.0567 mg/kg).

Table 4.5. Mercury concentrations in aquaculture farms for wet (November 2014) and dry season (February and April 2015.

BLOCK	Mean Mercury Concentration (ppm, mg/L)	Mercury Concentration Range (ppm, mg/L)
Block 1 (Eastern Bulacan)	0.0008	ND – 0.0034
Block 2 (Western Bulacan)	0.0004	ND – 0.0022
Block 3 (Northern Bataan)	0.0005	ND – 0.0024
Block 4 (Southern Bataan)	0.0010	ND – 0.0037
Block 5 (Cavite)	0.0022	ND – 0.0065
Block 7 (Pampanga)	0.0006	ND – 0.0014



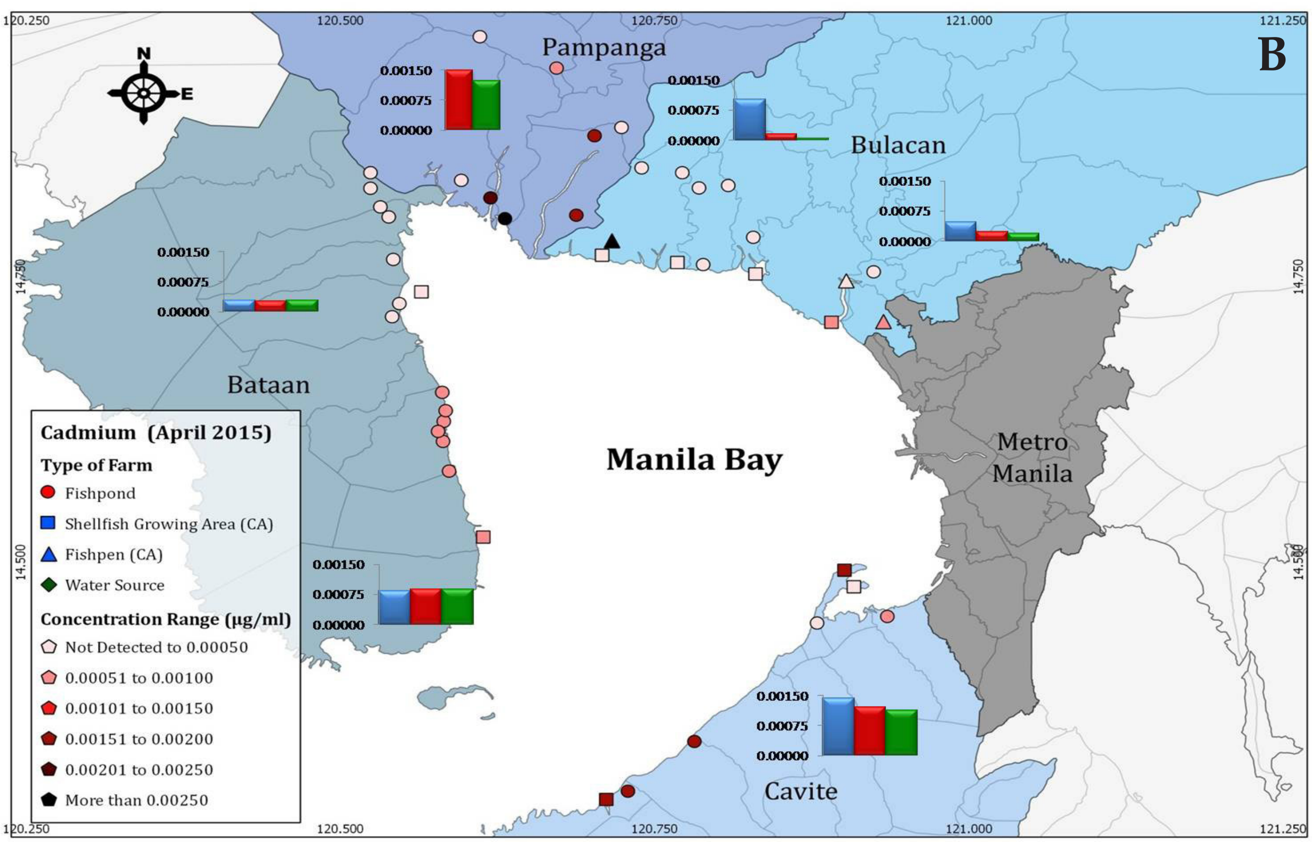


Figure 4.2. Levels of cadmium in coastal areas/fish pens, ponds, and water sourcesin November 2014 (A) and April 2015 (B).

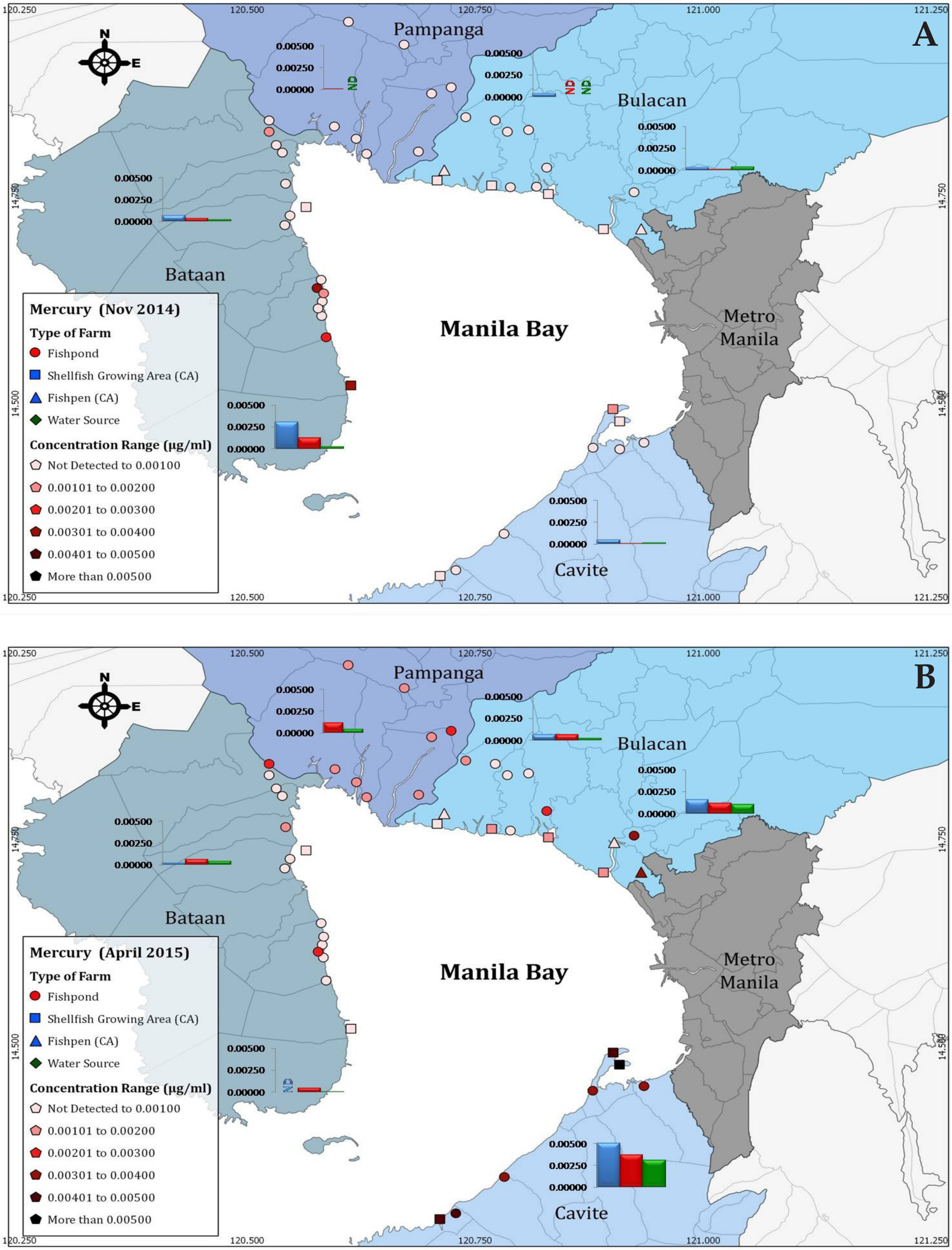


Figure 4.3. Levels of mercury in coastal areas/fish pens, ponds, and water sourcesin November 2014 (A) and April 2015 (B).

Table 4.6. Seasonal comparison of heavy metal concentrations in crab.

SEASON	HEAVY METAL CONCENTRATIONS (ppm, mg/kg)		
	Cd	Hg	Pb
Wet	0.0174	0.0122	0.0609
Dry	0.0191	0.0581	0.2640
	P = 0.997	P = 0.570	P = 0.030
Significance, $\alpha_{0.05}$	Not Significant	Not Significant	Significant

Table 4.7. Seasonal comparison of heavy metal concentrations in milkfish.

SEASON	HEAVY METAL CONCENTRATIONS (ppm, mg/kg)		
	Cd	Hg	Pb
Wet	0.0159	0.1568	0.1986
Dry	0.0105	0.0269	0.1384
	P = 0.068	P = 0.699	P = 0.788
Significance, $\alpha_{0.05}$	Not Significant	Not Significant	Not Significant

Table 4.8. Seasonal comparison of heavy metal concentrations in mussel.

SEASON	HEAVY METAL CONCENTRATIONS (ppm, mg/kg)		
	Cd	Hg	Pb
Wet	0.0269	0.0179	0.1781
Dry	0.0384	0.0844	0.2079
	P = 0.546	P = 0.534	P = 0.761
Significance, $\alpha_{0.05}$	Not Significant	Not Significant	Not Significant

Table 4.9. Seasonal comparison of heavy metal concentrations in oyster.

SEASON	HEAVY METAL CONCENTRATIONS (ppm, mg/kg)		
	Cd	Hg	Pb
Wet	0.0804	0.5590	0.1203
Dry	0.2044	0.2249	0.1515
	P = 0.153	P = 0.449	P = 0.663
Significance, $\alpha_{0.05}$	Not Significant	Not Significant	Not Significant

Table 4.10. Seasonal comparison of heavy metal concentrations in shrimp.

SEASON	HEAVY METAL CONCENTRATIONS (ppm, mg/kg)		
	Cd	Hg	Pb
Wet	0.0158	0.0076	0.0914
Dry	0.0091	0.0091	0.1652
	P = 0.327	P = 0.810	P = 0.654
Significance, $\alpha_{0.05}$	Not Significant	Not Significant	Not Significant

Table 4.11. Seasonal comparison of heavy metal concentrations in tilapia.

SEASON	HEAVY METAL CONCENTRATIONS (ppm, mg/kg)		
	Cd	Hg	Pb
Wet	0.0122	0.1021	0.1242
Dry	0.0121	0.0637	0.2223
	P = 0.978	P = 0.776	P = 0.205
Significance, $\alpha_{0.05}$	Not Significant	Not Significant	Not Significant

LEAD

Results for lead concentration in aquaculture commodities collected are reflected in Figure 4.4. Lead concentrations in samples collected in the wet season ranged from ND to 1.0723 mg/kg, with the highest noted in a milkfish sample from Eastern Bulacan, while dry season lead concentrations ranged from ND to 0.3953 mg/kg, the highest noted in a mussel sample from Cavite.

Lead concentrations in milkfish samples ranged from ND to 1.0723 mg/kg, highest in a sample collected in Eastern Bulacan, while lead levels in tilapia samples ranged from ND to 0.3087 mg/kg, highest in sample from Pampanga. Two out of twelve milkfish samples, collected from Eastern Bulacan (wet season) and Pampanga (dry season), and one out of nine tilapia samples, collected from Pampanga (dry), failed to meet the regulatory limit of 0.3 mg/kg (EC 1881/2006).

Crab samples had lead concentrations ranging from ND to 0.2688 mg/kg, highest in sample collected from Cavite while shrimp samples had lead levels that ranged from ND to 0.3065 mg/kg, highest in sample collected from Pampanga. Lead concentrations in all the crustacean samples were within the regulatory limit of 0.5 mg/kg (EC 1881/2006).

Lead in oyster and mussel samples ranged from ND to 0.3147 mg/kg and 0.0726 to 0.3953 mg/kg, respectively, both maximum values noted in Cavite. All oyster and mussel samples collected had lead concentrations within the regulatory limit of 1.5 mg/kg (EC 1881/2006).

CADMIUM

Cadmium levels in aquaculture commodities collected during the wet and the dry seasons are presented in Figure 4.5. Cadmium concentrations in the dry season (range of 0.0034 - 0.5526 mg/kg) were relatively higher compared to cadmium concentrations in wet season (range of ND - 0.1283 mg/kg). Highest concentrations for both seasons were noted in oyster samples from Western Bulacan.

Cadmium in milkfish samples ranged from ND to 0.0178 mg/kg, the highest concentration noted in a sample collected from Pampanga, while the levels in tilapia ranged from ND to 0.0191 mg/kg, the highest observed in a sample from Northern Bataan. All finfish samples had levels within the regulatory limit of 0.05 mg/kg (EC 1881/2006).

Crab and shrimp samples had cadmium concentrations ranging from 0.0047 to 0.0392 mg/kg and 0.0034 to 0.0158 mg/kg, respectively. The highest levels in both species were noted in samples collected from Pampanga. Cadmium concentrations in crustacean samples were within the regulatory limit of 0.5 mg/kg (EC 1881/2006).

Cadmium levels in oyster samples,

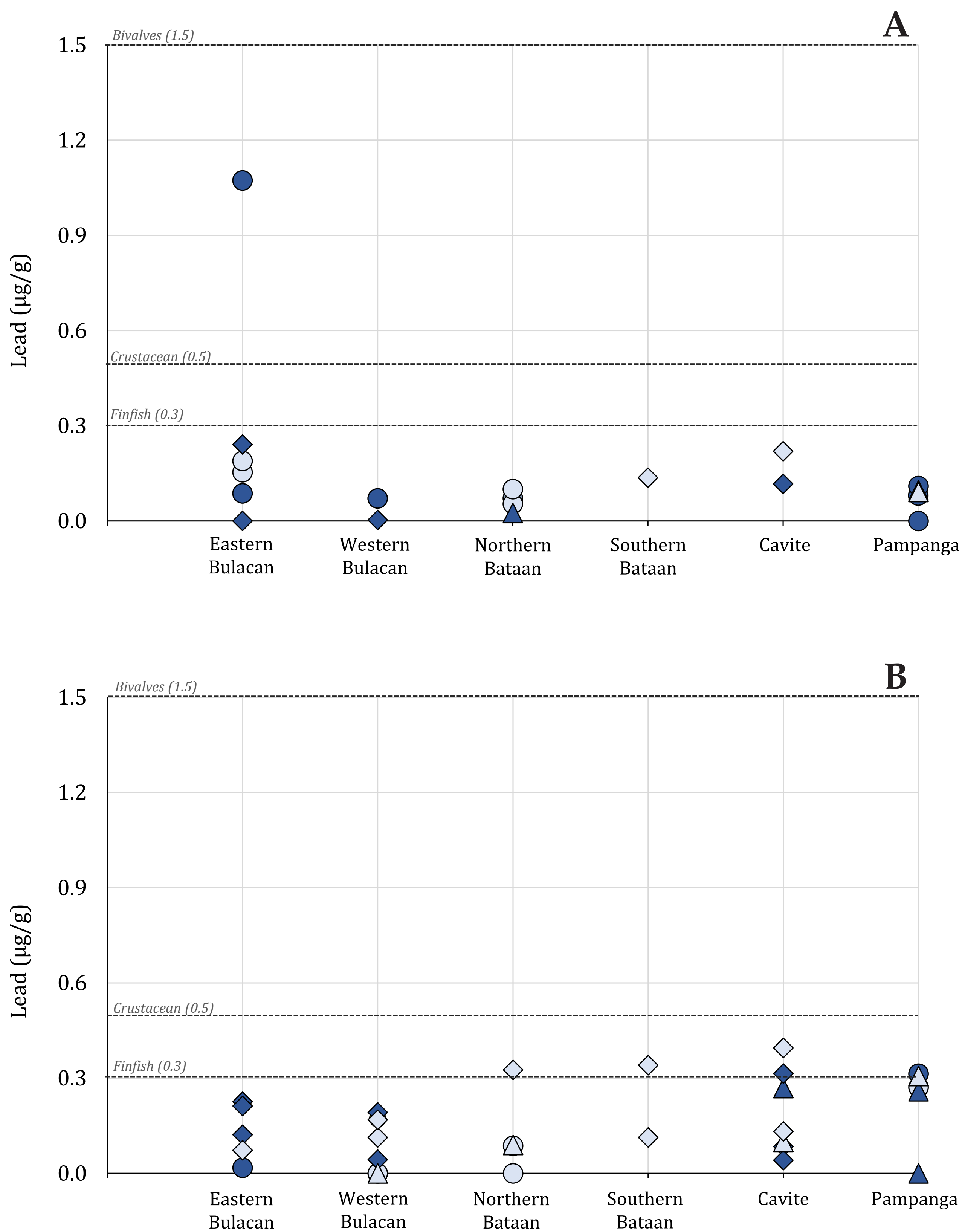
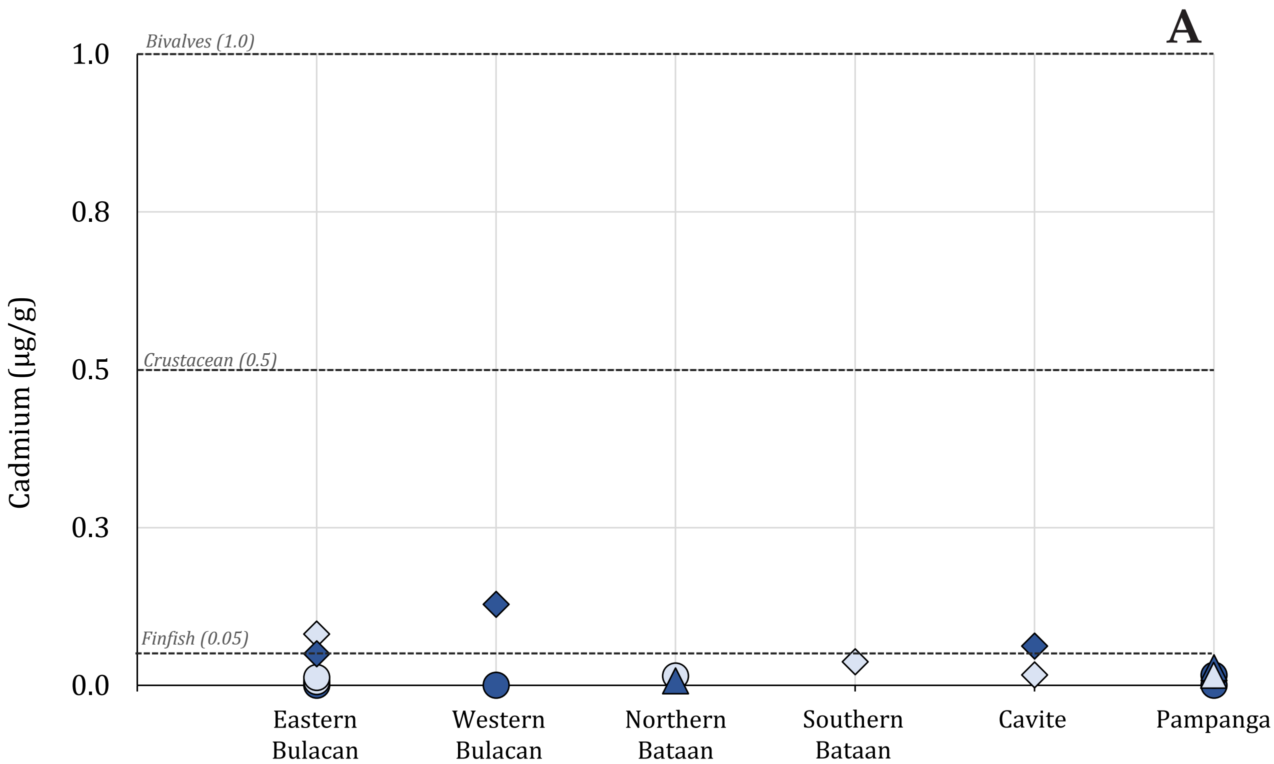


Figure 4.4. Lead levels in aquaculture commodities collected in wet season (A) and in the dry season (B).
Milkfish (MF) Tilapia (TI) Crab (CR) Shrimp (SH) Oyster (OY) Mussel (MU)



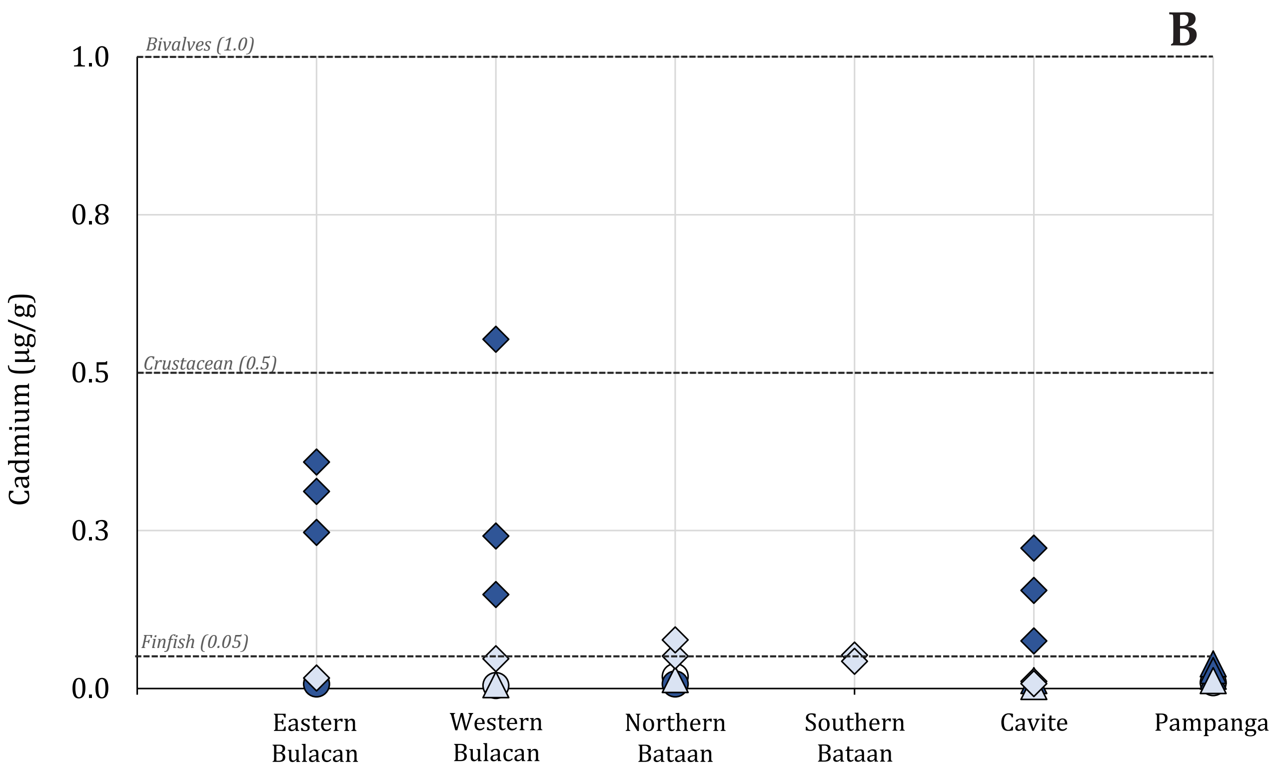


Figure 4.5. Cadmium levels in aquaculture commodities collected in the wet season (A) and in the dry season (B).
Milkfish (MF) Tilapia (TI) Crab (CR) Shrimp (SH) Oyster (OY) Mussel (MU)

ranging from 0.0500 to 0.5526 mg/kg, were highest in a sample collected in Western Bulacan. Oyster had higher levels than mussels whose range was from 0.0077 to 0.0769 mg/kg, and highest in a sample from Northern Bataan. All bivalve samples had cadmium concentrations within the regulatory limit of 1.0 mg/kg (EC 1881/2006).

MERCURY

Mercury in aquaculture commodities collected in the wet and the dry seasons are presented in Figure 4.6. As shown, the wet season samples had mercury levels ranging from ND to 1.1063 mg/kg, while dry season samples had levels ranging from ND to 0.1271 mg/kg. Maximum mercury concentrations were noted in oyster (wet) and mussel (dry) samples both collected from Eastern Bulacan.

Milkfish samples had mercury levels ranging from ND to 0.6662 mg/kg, highest in a sample collected from Pampanga, while tilapia samples had mercury concentrations ranging from ND to 0.2930 mg/kg, highest in sample collected from Eastern Bulacan. One out of twelve milkfish samples (collected from Pampanga, wet season) failed to meet the regulatory limit of 0.5 mg/kg (EC 1881/2006).

Mercury in crab samples ranged from ND to 0.1257 mg/kg, highest in a sample collected from Western Bulacan, while those in shrimp ranged from ND to 0.0846 mg/kg, highest in a sample collected from Northern Bataan. All crustacean samples had mercury levels within the regulatory limit of 0.5 mg/kg (EC 1881/2006).

Oyster and mussel samples had mercury concentrations ranging from ND to 1.1063 mg/kg and ND to 0.1271 mg/kg, respectively. Maximum values in both were noted in Eastern Bulacan. One out of thirteen oyster samples (collected from Eastern Bulacan, wet season) failed to meet the regulatory limit of 0.5 mg/kg (EC 1881/2006).

Method Validation

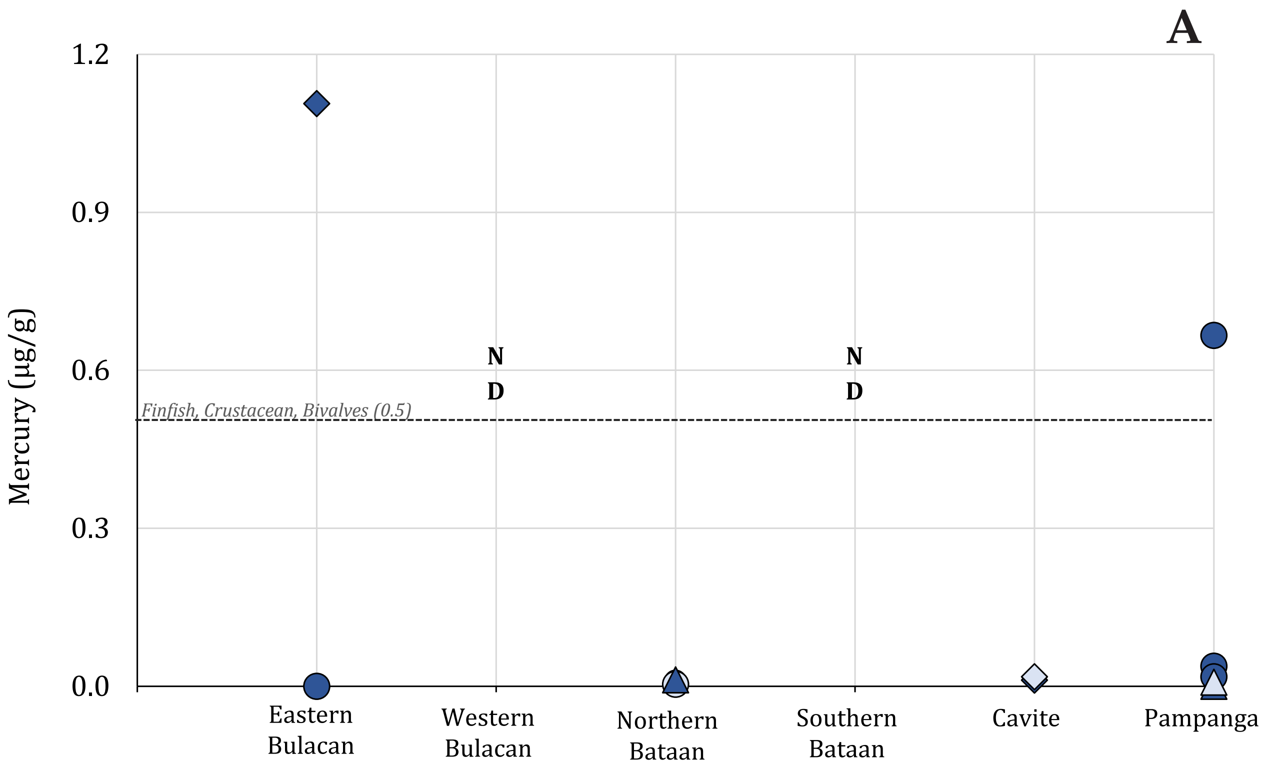
The methods for the determination of total cadmium (Cd), lead (Pb), and mercury (Hg) were validated by using spiked concentrations of 1 μg/L Cd, 5 μg/L Pb, and 0.1 μg Hg in different fishery resources such as finfish (n=9), crustaceans (n=7), and mollusk (n=9). Validation were also done in certified reference material (CRM) sample, DORM-3 (NRC-CNRC Fish Protein Certified Reference Material for Trace Metals) with certified values of 0.290±.020 mg/kg for cadmium, 0.395±0.050 mg/kg for lead, and 0.382±0.060 mg/kg for mercury. The calculations for the method detection limit (MDL) and limit of quantification (LOQ) were based on signal-to-noise ratio since the analytical procedure exhibited a baseline noise. The determination of method MDL and LOQ was done by comparing the measured signal of the spiked blank sample with those of the signal of the blank sample alone. Results of the method validation are summarized in Table 4.12.

Discussion

Spatial and Seasonal Distribution of Heavy Metals in Water

Heavy metals are known to exist in trace amounts in the environment. These are naturally harmless (Shremati & Varma, 2010). However, continuous urbanization and industrialization along with the increase in population and different anthropogenic activities result in the increment of these (Oluyemi et al, 2008) to alarming levels necessitating that regulatory standards are to be set.

Rainfall causes urban runoff, a non-



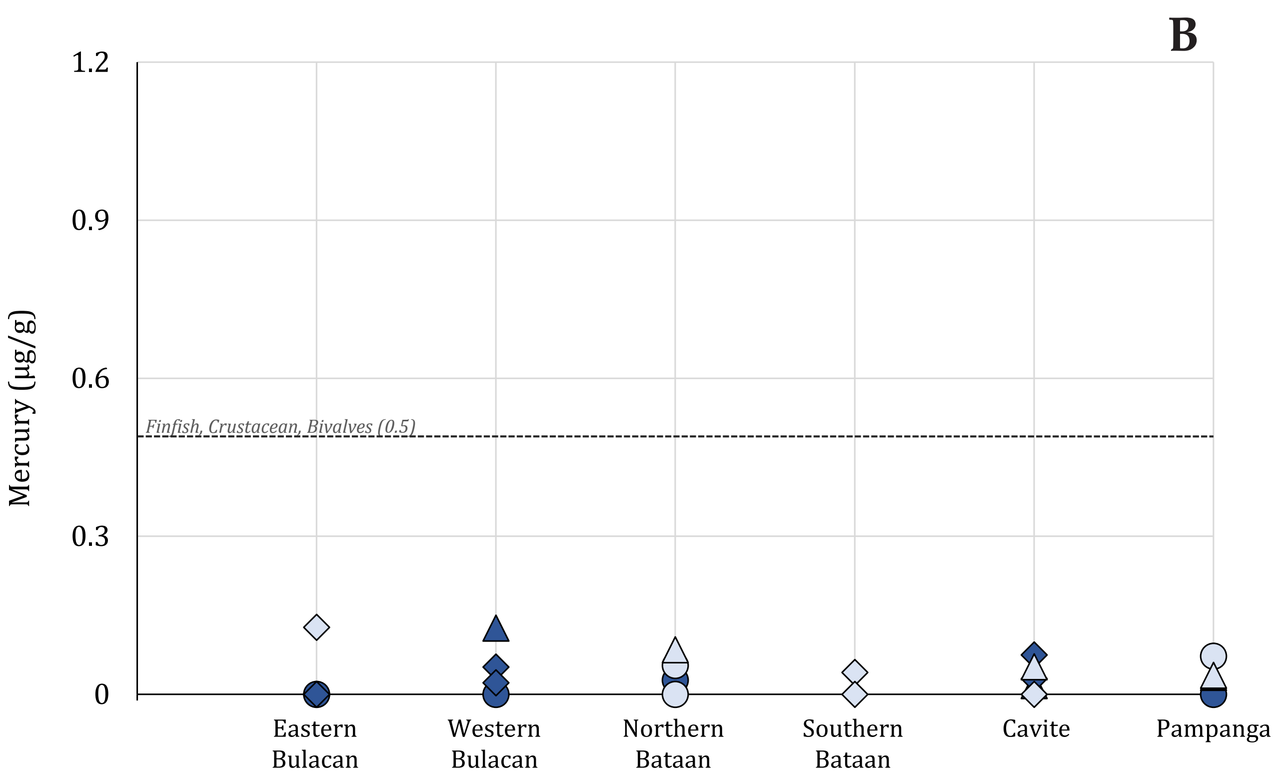


Figure 4.6. Mercury levels in aquaculture commodities collected in the wet season (A) and in the dry season (B).
● Milkfish (MF) ○ Tilapia (TI) ▲ Crab (CR) △ Shrimp (SH) ◆ Oyster (OY) ◇ Mussel (MU)

Table 4.12. Summary of the method validation results for total cadmium, lead, and mercury.

Performance Characteristics and its Accepted Criteria	**CADMIUM:** Microwave digestion followed by Graphite Furnace	**LEAD:** Microwave digestion followed by Graphite Furnace	**MERCURY:** Microwave digestion followed by Mercury Vapor Unit
MDL *	0.0011 (Fish) 0.0033 (Mollusk) 0.0034 (Crustaceans)	0.0334 (Fish) 0.0480 (Mollusk) 0.0205 (Crustaceans)	0.0353(Fish) 0.0291 (Bivalves)
LOQ ¥	0.0036 (Fish) 0.0109 (Mollusk) 0.0113 (Crustaceans)	0.1114 (Fish) 0.1598 (Mollusk) 0.0683 (Crustaceans)	0.1176 (Fish) 0.971 (Bivalves/ Mollusk)
Mean Concentration $^{\delta}$	0.0001±0.00002	0.0005±0.00007	
Mean % RSD $^{\alpha}$	1.68%	12.98%	
Accuracy: Spike % Recovery (40-120% for Pb and Cd; 80-110 for Hg) $^{\beta}$	87.49% 78.46-101.77%	77.06% 72.06-81.17%	76.7-113.4%
Accuracy: CRM % Recovery (80 -110%) $^{\beta}$	100.10% 92.81-106.65%	98.47% 88.39-110.73%	80.47-116.62% (Day 1) 88.38-125.84% (Day2)

All concentrations are expressed as µg/g total cadmium, lead and mercury

* *MDL is calculated as 3 X standard deviation*

¥ *LOQ is calculated as 3.33 X LOD*

δ *Mean concentration is the average concentration of the number of samples analyzed*

α *% Relative standard deviation, calculated as: (standard deviation divided by mean concentration) X 100*

β *% Recovery is calculated as: (difference of concentration of the spiked and unspiked samples divided by the theoretical concentration) X 100; reported % recovery range corresponds to number of samples analyzed.*

point source of pollution, that carries several contaminants, including heavy metals (US EPA, 2016), draining into river systems and enters aquaculture farms through water exchange activities (Srivanasa *et al*, 2007). It is known that water exchange activity in ponds is more frequent during the wet season since water levels in river tributaries or water sources increases with rainfall, thus more water is available for the farmers (Yoo& Boyd, 1994). This only means that rainfall results in an increase in heavy metal concentration in pond water due to the continuous influx of water. This is evident in the high concentrations of lead during the wet season where rainfall is greater.

Contrary to the case of lead, cadmium and mercury concentrations were found higher during the dry season. This can be attributed to slow water exchange owing to the limited supply from water sources, and/or due to an increase in temperature, which increases the evaporation rate. In a study by Nartey *et al* (2011), mercury concentrations in the river increased with increased evaporation rate of surface water. Cadmium, on the other hand, is a very soluble heavy metal easily leached from the sediments and dissolved in water (Rajan *et al*, 2013).

Aside from the observed seasonal distribution of heavy metal contamination in aquaculture farms, spatial distribution was also observed. Cadmium was found highest in Pampanga, where farming, fishing, manufacturing, handicrafts, poultry and swine, food processing industries, as well as ceramics and metalworking are the main sources of livelihood (pampanga.gov.ph). Wetlands, swamplands, agricultural lands, clay, gravel, sand, and copper are the natural resources there (alviera.ph). Pampanga is adjacent to Zambales, which houses Mount Pinatubo, an active volcano. Volcanic action is known as one of the natural sources of cadmium not only in the atmosphere but also in the soil, which in turn gets washed off into river systems (Buat-Ménard *et al*, 1987; Hutton *et al*, 1987). Quarrying and coal combustion, two of the anthropogenic activities that emit cadmium, are practiced in the province following the 1991 eruption of Mount Pinatubo as reported by the Greenpeace Southeast Asia and Orejas in 2014. Some aquaculture farmers do not observe proper buffer zone nor monitor water quality; they had the highest population of chicken and wild birds among the provinces, and used chicken manure and urea as fertilizers, as reported in the previous study conducted by the group, *"Review of Aquaculture Practice and Anthropogenic Activities in Manila Bay Aquaculture Farms."* Inorganic fertilizers such as urea as well as chicken manure may contain trace metals such as cadmium, which are deposited in pond sediment (Benson *et al*, 2014; Boyd & Masaut, 1999). Buffer zones filter out wastes, fertilizers, pesticide runoffs, and other contaminants (chemical and microbiological) from entering the ponds (DeFries, Karanth, & Pareeth, 2010). The lack of these may result in higher contamination of the pond.

Mercury was found highest in Cavite where the population is highest among the provinces sampled (NSO, 2010). Mercury is primarily used in dental amalgams, fluorescent lights, thermometers, electric switches, batteries, insecticide, disinfectant, rat poisons, and even in skin ointments (The Columbia Electronic Encyclopedia, 2012). Since the province has the highest population, it is safe to assume that production as well as usage of above-mentioned products is highest in the province and so is mercury emission. Aside from anthropogenic activities already discussed, mining and quarrying, huge contributors to mercury contamination, are major components of production land-use in the province (cavite.gov.ph). Mercury is discharged into the air (gets deposited into the ground through precipitation) or water through point sources (Lindberg *et al*, 1987). This in turn gets into aquaculture farms by way of river systems. In addition, aquaculture farmers in Cavite bypass residual waste removal and water flushing – two activities that reduce contamination in the pond – during pond preparation as reported in the previous study conducted by the group entitled, *"Review of Aquaculture Practice and Anthropogenic Activities in Manila Bay Aquaculture Farms."*

Lead, which is known to come from gasoline, batteries, ammunition, paint, ceramics, and even in cosmetics (US EPA, 2015) was found highest in Bataan, where oil and gasoline companies, refineries, and feed mills are the major industry sectors. The province, which will soon be considered a special defense economic zone, has also been manufacturing ammunition for nearly 60 years (Rivera, 2015). Aquaculture farmers in the province use urea, an inorganic fertilizer that may contribute to the concentration of lead in the water. In a study by Benson *et al* in 2013, lead concentration was found highest among the other trace elements in the urea fertilizer.

Heavy Metals Concentration in Fish and Fishery Resources

Aquaculture commodities such as milkfish, tilapia, shrimp, crab, mussel, and oyster differ in several ways – size, production cycle, feeding habit, etc. It is only logical to assume that the uptake of heavy metals in the above-mentioned commodities differ from species to species. In fact, findings in a study by Su *et al* (2009) suggest that accumulation of heavy metals in aquatic organisms is species-dependent. In the present study, heavy metal accumulation in aquaculture commodities is as follows: oyster > mussel >tilapia > shrimp > crab > milkfish.

Heavy metal contamination in bivalves, which was observed to be higher compared to the other aquaculture commodities analyzed, may be attributed to their feeding habit of filter feeding (Garrido-Handog, 1990; Aypa, 1990). Filter feeders siphon the water in which they are suspended through filters that retain suspended matter, like phytoplanktons (Barker Jørgensen, 1990). This siphoning and filtration processes cause heavy metals in the water column to pass through the organism, ultimately to get deposited in different cellular compartments (Rodríguez de la Rúa *et al*, 2005). Oysters and mussels were collected from coastal areas. It is known that all effluents, treated and untreated, flow to the bay as the bay is the catch basin for all water systems in Manila Bay. As shown in the results on the heavy metals in water, although inconsistent, concentration of heavy metals came out to be high in coastal areas. This implies that higher heavy metal concentration in the water column causes a higher contamination in aquaculture commodities.

Heavy metals in aquaculture commodities were found higher during the dry season than the wet season. Milkfish, tilapia, shrimp, and crab feeding habits depend on temperature; as temperature increases, feeding frequency also increases (FAO[2], 2011; Garcia, 1990; Greenfield *et al*, 2005; Benitez, 1984) and so does their heavy metal uptake.

Conclusion

Lead was found higher during the wet season while cadmium and mercury, during the dry season. The following are the sites that failed to meet DENR regulatory limits for heavy metals in water in wet season: Northern Bataan (1) and Southern Bataan (2) for lead (0.05 µg/mL); Northern Bataan (3) for mercury (0.002 µg/mL); while the following are the sites that failed DENR regulatory limits in the dry season: Eastern Bulacan (3), Western

Bulacan (1), Northern Bataan (1), Southern Bataan (1), and Cavite (8) for mercury (0.002 µg/mL). Cadmium was found highest in Pampanga, while mercury and lead in Cavite and Bataan, respectively. Two out twelve milkfish samples and one out of nine tilapia samples failed to meet the regulatory limit for lead in finfish (0.3µg/g) while one out of twelve milkfish samples and one out of thirteen oyster samples exceeded the limit for mercury in finfish (0.5 µg/g) and bivalves (0.5 µg/g), respectively.

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