

# Bioaccumulation and Non-carcinogenic Health Risk Assessment of Heavy Metals in Selected Fish Species from South Bay of Laguna Lake

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## ABSTRACT

**Objective.** The study aimed to determine the concentration of three heavy metals namely, lead (Pb), cadmium (Cd) and chromium (Cr) present in head, stomach and muscle parts of Nile Tilapia (*Oreochromis niloticu*) and Blackchin Tilapia (*Sarotherodon melanotheron*) collected from different sampling sites in South Bay area of Laguna Lake and evaluate its potential health risk for adult consumers.

**Methods.** Live samples of Nile Tilapia and Blackchin Tilapia were hand-collected from three sites of South Bay of Laguna Lake during wet season. Raw and boiled samples were prepared for analysis. Heavy metal concentrations (mg kg<sup>-1</sup>) were determined through flame atomic absorption spectrophotometry (AAS). The potential health risk posed by more than one metal was calculated using the Total Hazard Quotient (THQ).

**Results.** The heavy metal detected with the highest concentration was Pb, present in Nile Tilapia. The mean concentrations of Pb, Cd, and Cr in both fish samples were high in the head part. Boiled samples have significantly lower concentrations of heavy metal ( $p < 0.05$ ) compared to raw samples. THQ values for Cd in raw fish samples were higher than the recommended level of exposure (THQ < 1.0).

**Conclusion.** The study revealed that cadmium is of greater concern in terms of possible adverse health effects associated with heavy metal bioaccumulation. However, the presence of other metals may further increase the risk of non-carcinogenic adverse effects. The lower health risk is predicted for boiled fish samples.

**Key Words:** Health risk, Heavy metals, Total hazard quotient (THQ), Hazard Index (HI), Nile Tilapia, Blackchin Tilapia

## INTRODUCTION

Laguna Lake is the largest lake in the Philippines and the second largest in Southeast Asia. The most dominant use of the lake is on fisheries. From 1997 to 2000, the lake yielded approximately 37,000 to 47,000 MT (metric tons) of fish— both from the fish pens and open fisheries.<sup>1</sup> It has been known as a major source of food and livelihood for people inhabited around it. However, Laguna Lake is besieged daily with anthropogenic pollutants from industrial, domestic and agricultural waste which can be absorbed by aquatic plants and animals. According to Molina (2011; 2015), the Laguna Lake water quality has deteriorated through the years due to various point sources of pollution.<sup>2</sup> Moreover, industry development and rapid urbanization from the northwestern to the western shores of Laguna

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Lake have led to land-use changes which result in water pollution and ecosystem degradation.<sup>3</sup>

The presence of pollutants causes undesirable changes in an ecosystem. This has both a direct and indirect impact on the ecological balance of the environment and ultimately affects humans. Among these numerous contaminants, heavy metal pollution in coastal regions has become a global threat because of its toxicity.<sup>4</sup> As a result, aquatic organisms become exposed to elevated levels of heavy metals.<sup>5,6</sup>

Eight common heavy metals are arsenic, barium, cadmium, chromium, lead, mercury, selenium, and silver. These are all naturally occurring substances which are often present in the environment at low levels but can be dangerous in larger amounts.<sup>7</sup>

Results of many field studies of metal accumulation in fish living in polluted waters show that considerable amounts of various metals deposit in fish tissues without causing mortality.<sup>4</sup> Molina (2011) reported that commonly consumed fish products from Laguna lake (i.e. Bangus, Bighead Carp, Dalag, Kanduli, and Tilapia) were contaminated with Cadmium (Cd), Lead (Pb), Mercury (Hg), Arsenic (As), and Chromium (Cr). The results of the study also further revealed that concentrations of heavy metals in fish samples varied from different seasons. There was generally lower heavy metal concentration during wet season compared to dry season.<sup>2</sup>

Humans are generally exposed to heavy metals through ingestion or inhalation. Health Risk Assessment is done to determine the likelihood of harm associated with exposure to the chemical (WHO, 2010).<sup>8</sup> This study aimed to evaluate the existing levels of heavy metals such as chromium, cadmium, and lead in two edible fish species (i.e., *Nile Tilapia* and *Blackchin Tilapia*) collected from South Bay area of Laguna Lake and predict potential health implications of long-term consumption heavy metal-contaminated fish samples.

## MATERIALS AND METHODS

### Sample collection

Three coastal areas adjacent to towns around the South Bay area of Laguna Lake (i.e., Calamba, Los Baños and Bay, Laguna) were chosen as collection sites (Figure 1). Live samples of Nile Tilapia (*Oreochromis niloticu*) and Blackchin Tilapia (*Sarotherodon melanotheron*) were hand-collected from each site with the help of professional local fishermen during the wet season (June 2017). All samples were placed into dark plastic bags containing the ambient water and transported to the laboratory in an iced chest. Three edible parts (i.e. head, stomach, and muscle tissue) of both Nile and Blackchin Tilapia were separated for individual analysis. Two sets of samples were prepared, one of which was raw while the other was boiled. All pooled samples were analyzed for Pb, Cd and Cr concentration.

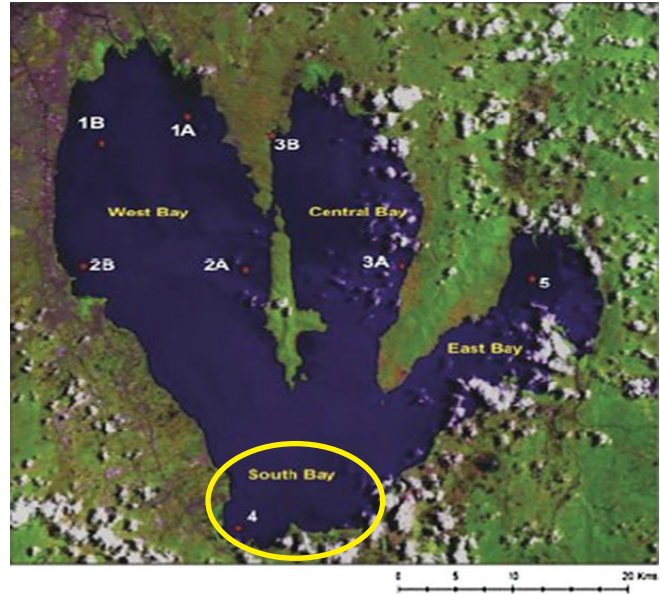


Figure 1. Sample Location (Source: Molina, 2014).

### Heavy Metal Concentration Analysis

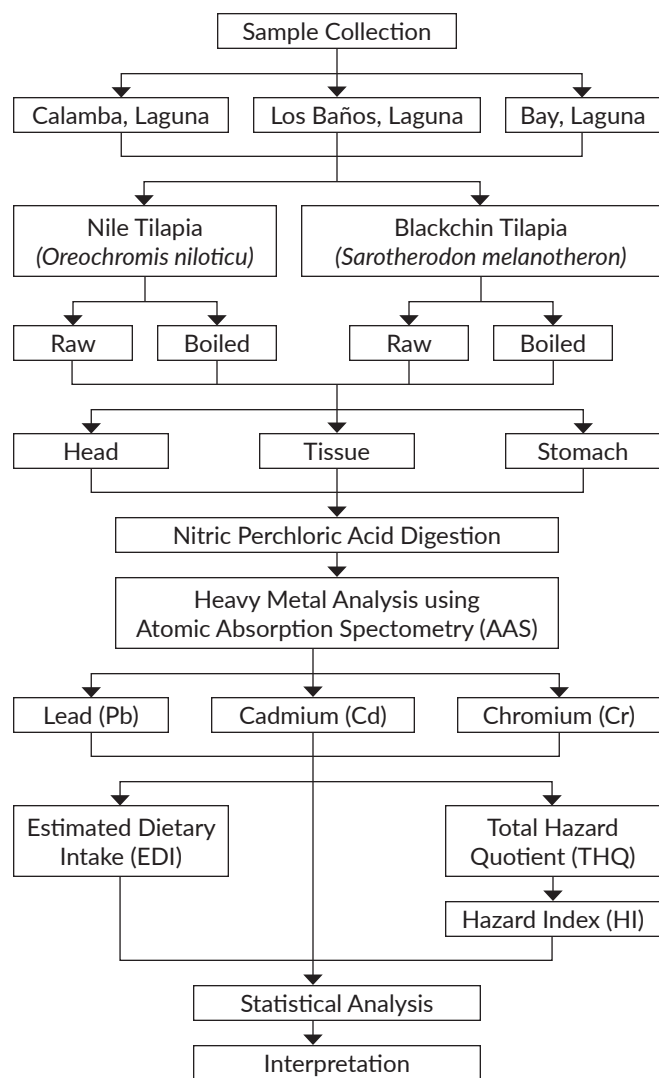
The samples (both raw and boiled) were homogenized before wet digestion using Nitric-perchloric acid digestion (Figure 2), following the procedure recommended by the AOAC (1990).<sup>9</sup> Boiled samples were cooked using distilled water for 10 minutes. Five grams of sample was placed in a 250 mL digestion tube and 15 mL of concentrated Nitric acid (HNO<sub>3</sub>) was added. The mixture was heated gently for 30–45 min to oxidize all easily oxidizable matter. After cooling, 5 mL of 70% perchloric acid (HClO<sub>4</sub>) was added to the mixture then heated gently until dense white fumes appeared. After cooling, 20 mL of distilled water was added, and the mixture was heated further to release any fumes. The solution was cooled and filtered through Whatman No. 42 filter paper. Total heavy metal concentration (in mg kg<sup>-1</sup>) was determined by flame atomic absorption spectrometry (SPAA-3000 SPECTRUM Instrument) fueled with air acetylene using 217 nm spectral wavelength. The detection limit for [Pb], [Cd] and [Cr] were determined at 0.004, 0.002 and 0.001 mg per liter respectively. All laboratory experiments were performed at Laguna State Polytechnic University-Los Baños Campus, Science Research Laboratory.

### Health Risk Assessment

The estimated daily intake (EDI) of Pb, Cd, and Cr in fish samples were estimated using the following equation:<sup>10</sup>

$$[EDI = IR \times MC / BW]$$

where, IR is the ingestion rate of the samples (102.74 g day<sup>-1</sup>, this is the estimated average daily per capita consumption of fish in the Philippines according to FAO- Fisheries and Aquatic Department),<sup>11</sup> MC is the metal concentration



**Figure 2.** Study Protocol.

of Cd, Cr, and Pb in samples (in milligrams per kilogram, fresh weight), and BW is the average human body weight (52.5 kg for female and 60.5 kg for male, according to Philippine Dietary Reference Intake, 2015).<sup>12</sup> To assess the non-carcinogenic risk due to Cd, Cr, and Pb exposure in fish samples, the target hazard quotient (THQ) was computed using the following equation:<sup>13</sup>

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

where, EFr is exposure frequency (number of days per year for the average and heavy consumer, assuming 365 days/ year); ED is exposure duration (68 years for Filipino) equivalent to the average human lifespan, IR is the ingestion rate of fish (102.74 g day<sup>-1</sup>, this is the estimated average daily per capita consumption of fish in the Philippines according to FAO- Fisheries and Aquatic Department,<sup>11</sup>; MC is the metal concentration of Cd, Cr, and Pb in samples (in milligrams per kilogram, fresh weight); RfDo is the oral

reference dose. The applied reference doses according to USEPA (2011) are the following: 0.2 mg kg<sup>-1</sup> day<sup>-1</sup> for Pb; 0.005 mg kg<sup>-1</sup> day<sup>-1</sup> for Cd; 0.009 mg kg<sup>-1</sup> day<sup>-1</sup> for Cr.<sup>14</sup> BW is the average human body weight (52.5 kg for female and 60.5kg for male;<sup>12</sup> AT is the averaging time for non-carcinogens (assuming 30 years or 10,950 days).

To assess the potential health risk posed by more than one metal, THQ of every metal is summed up and it is known as the Hazard Index (HI). The HI was calculated using the sum of the target hazard quotients of each metal:<sup>15</sup>

$$HI = [THQ]Pb + [THQ]Cd + [THQ]Cr$$

### Statistical Analysis

Mean and standard deviations were used to describe the concentration of Cd, Cr, and Pb in the samples. Significant mean differences in heavy metal concentration among samples from different sampling zone and sample parts were tested using two factor ANOVA and pairwise comparison. Statistical tests were performed in SPSS version 20.0 at 95% confidence level.

## RESULTS

### Heavy Metal Concentration in the Three Sampling Sites

Table 1 shows the heavy metal concentration detected in the different sampling sites. The highest mean Pb concentration was detected in fish samples collected from Los Baños followed by samples collected from Calamba and Bay respectively. The result also revealed that the mean [Pb] in the samples from Los Baños (1.546±0.52 mg kg<sup>-1</sup>) were significantly different (p<0.05) from the mean [Pb] in samples from Calamba (1.199±0.42 mg kg<sup>-1</sup>) and Bay (1.065±0.31 mg kg<sup>-1</sup>)

For [Cd], it was recorded that the highest concentration was present in fish samples collected from Los Baños followed by Calamba and Bay. Statistical comparisons indicate that the mean [Cd] in samples from Los Baños (0.177±0.09 mg kg<sup>-1</sup>) was significantly different (p<0.05) from the mean [Cd] in the fish samples from Calamba (0.119±0.02 mg kg<sup>-1</sup>) and Bay (0.093±0.04 mg kg<sup>-1</sup>), whereas, the mean [Cd] in the fish samples from Calamba was not significantly different from samples collected from Bay (P>0.05).

The mean concentration of Cr was measured highest in fish samples collected from Los Baños (0.051±0.02 mg kg<sup>-1</sup>) followed by Calamba (0.042±0.02 mg kg<sup>-1</sup>) and Bay (0.039±0.02 mg kg<sup>-1</sup>). The statistical comparison indicated that the mean [Cr] in samples from Los Baños was significantly different from the mean [Cr] in samples from Bay (p<0.05). Moreover, no significant differences (p>0.05) were found between the mean concentration of Cr in samples from Calamba and mean [Cr] in samples from Los Baños and Bay.

**Table 1.** Mean Heavy Metal concentration (mg kg<sup>-1</sup>, fresh weight) in fish samples collected from different sites

Sampling Sites	Lead (Pb) Mean ± SD	Cadmium (Cd) Mean ± SD	Chromium (Cr) Mean ± SD
Los Baños	1.546 ± 0.52 <sup>a</sup>	0.177 ± 0.09 <sup>a</sup>	0.051 ± 0.02 <sup>a</sup>
Calamba	1.199 ± 0.42 <sup>b</sup>	0.119 ± 0.02 <sup>b</sup>	0.042 ± 0.02 <sup>ab</sup>
Bay	1.065 ± 0.31 <sup>b</sup>	0.093 ± 0.04 <sup>b</sup>	0.039 ± 0.02 <sup>b</sup>

Values having the same superscript across a column are not significantly different at P<0.05

**Table 2.** Mean Heavy Metal concentration (mg kg<sup>-1</sup>, fresh weight) in Nile Tilapia and Blackchin Tilapia

Sampling Sites	Lead (Pb) Mean ± SD	Cadmium (Cd) Mean ± SD	Chromium (Cr) Mean ± SD
Nile Tilapia	1.1625 ± 0.58 <sup>a</sup>	0.47 ± 0.02 <sup>a</sup>	0.047 ± 0.02 <sup>a</sup>
Blackchin Tilapia	1.3778 ± 0.29 <sup>a</sup>	0.155 ± 0.05 <sup>b</sup>	0.041 ± 0.02 <sup>a</sup>

Values having the same superscript across a column are not significantly different at P<0.05

**Bioaccumulation of Heavy Metals in Fish Species**

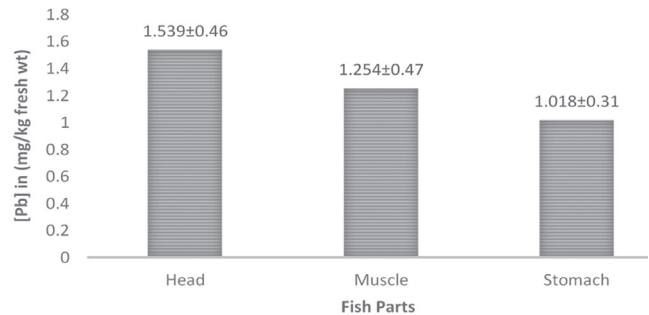
Table 2 presents the heavy metal accumulation in the two sampled fish species. Pb bioaccumulation in Blackchin Tilapia was higher than in Nile Tilapia. However, statistical comparison between the mean [Pb] in Blackchin Tilapia (1.38mg kg<sup>-1</sup>) was not significantly different (p<0.05) from the mean [Pb] in Nile Tilapia (1.16mg kg<sup>-1</sup>). The mean [Cd] was also higher in Blackchin Tilapia (0.155±0.05 mg kg<sup>-1</sup>) and is significantly different (p<0.05) from the mean [Cd] in Nile Tilapia (0.104±0.08 mg kg<sup>-1</sup>). Lastly, the mean [Cr] in Blackchin Tilapia (0.041±0.02 mg kg<sup>-1</sup>) is lower than the mean [Cr] in Nile Tilapia (0.047±0.02 mg kg<sup>-1</sup>) but the difference was not statistically significant at p<0.05.

Table 3 gives the values of heavy metal concentration in boiled fish compared to raw fish. The statistical comparison revealed that there were significant differences (p<0.05) between the mean concentrations of Pb, Cd, and Cr in raw and boiled fish species. In Nile Tilapia, boiling caused approximately 71% decrease in [Pb], 50% decrease in [Cd] and 60% decrease in [Cr]. On the other hand, in Blackchin Tilapia, boiling brought about a 69% decrease in [Pb], a 53% decrease in [Cd] and 75% decrease in [Cr].

**Heavy Metal Concentration from different parts of the Fish**

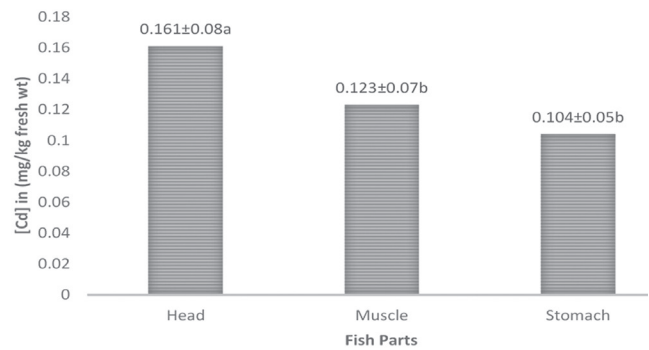
The mean Pb concentration in the head part (1.539±0.46 mg kg<sup>-1</sup>) was highest among the three parts followed by

the muscle (1.254±0.47 mg kg<sup>-1</sup>) and stomach (1.018±0.31 mg kg<sup>-1</sup>) in both fish samples (Figure 3). The statistical comparison showed that the mean [Pb] from three parts were significantly different from each other (p<0.05).



**Figure 3.** Mean Lead (Pb) concentration (mg kg<sup>-1</sup>, fresh weight) in the different fish parts.

Among the fish parts, the mean concentration of Cd was found highest in the head part (0.161±0.08 mg kg<sup>-1</sup>) followed by the muscle (0.123±0.07 mg kg<sup>-1</sup>) and stomach (0.104±0.05mg kg<sup>-1</sup>) in both fish samples (Figure 4). Statistical comparisons show that the mean Cd concentration in the head part was significantly different from both muscle and stomach (p<0.05). Meanwhile, the mean concentration of Cd in the muscle and stomach was not significantly different (p>0.05).



**Figure 4.** Mean Cadmium (Cd) concentration (mg kg<sup>-1</sup>, fresh weight) in the different fish parts.

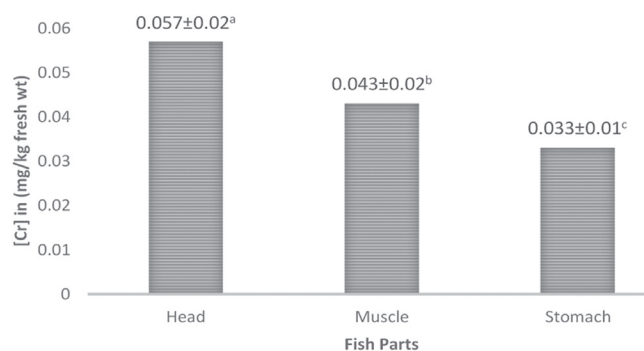
Cr bioaccumulation pattern in the fish parts was the same observed from Pb and Cd. The highest mean concentration of Cr was recorded in the head part (0.057±0.02 mg kg<sup>-1</sup>) followed by the muscle part (0.043±0.02 mg kg<sup>-1</sup>) and stomach (0.033±0.01 mg kg<sup>-1</sup>) (Figure 5). The statistical

**Table 3.** Means of Heavy Metal concentration (mg kg<sup>-1</sup>,fresh weight) in Raw and Boiled Nile and Blackchin Tilapia

Treatment	Lead (Pb)		Cadmium (Cd)		Chromium (Cr)	
	Nile	Blackchin	Nile	Blackchin	Nile	Blackchin
Raw	1.16±0.58 <sup>a</sup>	1.38±0.29 <sup>a</sup>	0.10±0.08 <sup>a</sup>	0.15±0.05 <sup>a</sup>	0.05±0.02 <sup>a</sup>	0.04±0.02 <sup>a</sup>
Boiled	0.34±0.25 <sup>b</sup>	0.43±0.37 <sup>b</sup>	0.05±0.02 <sup>b</sup>	0.07±0.03 <sup>b</sup>	0.02±0.0 <sup>b</sup>	0.01±0.00 <sup>b</sup>
% Reduction	70.89%	68.84%	40%	53.33%	60%	75%

Values having the same superscript across a column are not significantly different at P<0.05





**Figure 5.** Mean Chromium (Cr) concentration (mg kg<sup>-1</sup>, fresh weight) in the different fish parts.

comparison revealed that the mean [Cr] from three parts were significantly different from each other ( $p < 0.05$ ).

### Human Health Risk Assessment

The estimated dietary intake (EDI) of metals based on per capita consumption of fish in the Philippines are shown in Table 4. The EDI of Pb from the consumption of raw Nile Tilapia ranged from 1.48 mg kg<sup>-1</sup> bw day<sup>-1</sup> to 3.38 mg kg<sup>-1</sup> bw day<sup>-1</sup> with the highest Pb intake coming from Los Baños and lowest in Bay. In Blackchin Tilapia, EDI ranged from 2.11 mg kg<sup>-1</sup> bw day<sup>-1</sup> to 2.99 mg kg<sup>-1</sup> bw day<sup>-1</sup> with the highest intake coming from Calamba and lowest in Bay. Higher EDIs of Pb in both fish species were recorded in females compared to males. Essentially, lower EDI was obtained from boiled fish compared to raw since the previous result showed that there was a significant decrease in Pb concentration in the fish tissues when boiled. The EDI of Pb for boiled Nile Tilapia ranged from 0.21 mg kg<sup>-1</sup> bw day<sup>-1</sup> to 0.91 mg kg<sup>-1</sup> bw day<sup>-1</sup> while the EDI for boiled Blackchin Tilapia ranged from 0.21 mg kg<sup>-1</sup> bw day<sup>-1</sup> to 1.15 mg kg<sup>-1</sup> bw day<sup>-1</sup>. All EDI of Pb, except from the consumption of

boiled Blackchin Tilapia in Bay, exceeded the WHO/FAO maximum tolerable concentration (MTC) of 0.30 and indicates potential health risks to consumers.<sup>13,16</sup>

For the estimated daily intake (EDI) of Cd, all, except from the consumption of boiled Nile Tilapia in Los Baños, exceeded WHO/FAO maximum permissible limit of 0.05. This implies a potential adverse non-carcinogenic health risk to consumers. Regardless of the location, the EDIs ranged from 0.10 mg kg<sup>-1</sup> bw day<sup>-1</sup> to 0.31 mg kg<sup>-1</sup> bw day<sup>-1</sup> for raw Nile Tilapia while 0.22 mg kg<sup>-1</sup> bw day<sup>-1</sup> to 0.38 mg kg<sup>-1</sup> bw day<sup>-1</sup> for raw Blackchin Tilapia. The Highest EDI of Cd was recorded in Los Baños. Similar to Pb, EDIs of Cd for boiled samples were lower compared to raw. EDIs ranged from 0.03 mg kg<sup>-1</sup> bw day<sup>-1</sup> to 0.11 mg kg<sup>-1</sup> bw day<sup>-1</sup> in boiled Nile Tilapia and 0.10 mg kg<sup>-1</sup> bw day<sup>-1</sup> to 0.17 mg kg<sup>-1</sup> bw day<sup>-1</sup> in boiled Blackchin Tilapia.

Among the heavy metals studied, Cr concentration was found to have the lowest EDI in both fish samples. Results showed that none of the EDIs exceeded the maximum tolerable concentration of 1 mg kg<sup>-1</sup> bw day<sup>-1</sup>.<sup>16</sup> The EDI of Cr for raw Nile Tilapia and Blackchin Tilapia ranged from 0.077 mg kg<sup>-1</sup> bw day<sup>-1</sup> to 0.096 mg kg<sup>-1</sup> bw day<sup>-1</sup> and 0.053 mg kg<sup>-1</sup> bw day<sup>-1</sup> to 0.104 mg kg<sup>-1</sup> bw day<sup>-1</sup> respectively. For boiled Nile and Blackchin Tilapia, EDI of Cr ranged from 0.024 mg kg<sup>-1</sup> bw day<sup>-1</sup> to 0.036 mg kg<sup>-1</sup> bw day<sup>-1</sup> and 0.021 mg kg<sup>-1</sup> bw day<sup>-1</sup> to 0.027 mg kg<sup>-1</sup> bw day<sup>-1</sup> respectively. Highest EDI was recorded in Los Baños. Similar to Pb and Cd, lower EDI of Cr was obtained from the consumption of boiled samples compared to raw.

Table 5 provided information on the total hazard quotient (THQ) of metals from the consumption of these fish species. These parameters were introduced by the Environmental Protection Agency (EPA) in the United States for the estimation of potential health risks caused by any chemical contaminant over prolonged exposure.<sup>17</sup>

**Table 4.** Estimated dietary intake (EDI) of heavy metals in raw and boiled samples collected from different locations

Location	Specie	Treatment	EDI (mg kg <sup>-1</sup> bw day <sup>-1</sup> )					
			Pb		Cd		Cr	
			Male	Female	Male	Female	Male	Female
Los Baños	Nile Tilapia	Raw	2.94*	3.38*	0.27*	0.31*	0.083	0.096
		Boiled	0.71*	0.81*	0.03	0.03	0.031	0.036
	Blackchin Tilapia	Raw	2.32*	2.67*	0.33*	0.38*	0.091	0.104
		Boiled	1.00*	1.15*	0.10*	0.12*	0.023	0.026
Calamba	Nile Tilapia	Raw	1.48*	1.70*	0.16*	0.19*	0.077	0.089
		Boiled	0.79*	0.91*	0.10*	0.11*	0.025	0.029
	Blackchin Tilapia	Raw	2.59*	2.99*	0.24*	0.28*	0.066	0.076
		Boiled	0.98*	1.13*	0.11*	0.12*	0.021	0.024
Bay	Nile Tilapia	Raw	1.51*	1.74*	0.10*	0.12*	0.079	0.091
		Boiled	0.21*	0.24*	0.08*	0.09*	0.024	0.027
	Blackchin Tilapia	Raw	2.11*	2.43*	0.22*	0.25*	0.053	0.061
		Boiled	0.21	0.24	0.15*	0.17*	0.024	0.027
Maximum Permissible Limit			0.30 mg/kg <sup>a</sup>		0.05mg/kg <sup>a</sup>		1 mg/kg <sup>b</sup>	

\*Values that exceeded the Maximum Permissible Limit

<sup>a</sup>FAO/WHO (2011), <sup>b</sup>FAO/WHO (2002)

**Table 5.** Total Hazard Quotient (THQ) and Hazard Index (HI) for different heavy metals of two fish species collected from different locations at South Bay of Laguna Lake

Location	Specie	Treatment	THQ								
			Pb			Cd			Cr		
			Male	Female	Ave	Male	Female	Ave	Male	Female	Ave
Los Baños	Nile Tilapia	Raw	0.33	0.38	<b>0.36</b>	1.21*	1.39*	<b>1.30*</b>	0.21	0.24	<b>0.23</b>
		Boiled	0.08	0.09	<b>0.09</b>	0.13	0.16	<b>0.14</b>	0.08	0.09	<b>0.08</b>
	Blackchin Tilapia	Raw	0.26	0.30	<b>0.28</b>	1.51*	1.74*	<b>1.63*</b>	0.23	0.26	<b>0.25</b>
		Boiled	0.11	0.13	<b>0.12</b>	0.46	0.53	<b>0.50</b>	0.06	0.07	<b>0.06</b>
Calamba	Nile Tilapia	Raw	0.17	0.19	<b>0.18</b>	0.74	0.86	<b>0.80</b>	0.19	0.22	<b>0.21</b>
		Boiled	0.09	0.10	<b>0.10</b>	0.44	0.51	<b>0.48</b>	0.06	0.07	<b>0.07</b>
	Blackchin Tilapia	Raw	0.29	0.34	<b>0.32</b>	1.09*	1.25*	<b>1.17*</b>	0.17	0.19	<b>0.18</b>
		Boiled	0.11	0.13	<b>0.12</b>	0.49	0.56	<b>0.52</b>	0.05	0.06	<b>0.06</b>
Bay	Nile Tilapia	Raw	0.17	0.20	<b>0.18</b>	0.46	0.53	<b>0.50</b>	0.20	0.23	<b>0.21</b>
		Boiled	0.02	0.03	<b>0.03</b>	0.36	0.41	<b>0.39</b>	0.06	0.07	<b>0.06</b>
	Blackchin Tilapia	Raw	0.24	0.28	<b>0.26</b>	0.98	1.12*	<b>1.05*</b>	0.13	0.15	<b>0.14</b>
		Boiled	0.02	0.03	<b>0.03</b>	0.68	0.78	<b>0.73</b>	0.06	0.07	<b>0.06</b>

\*Values are higher than the recommended limit (THQ  $\geq$  1.0)

For Pb, all THQ value was less than 1.0. The highest average THQ for male and female were estimated in raw Nile Tilapia from Los Baños with a score of only 0.36. Hence, adverse non-carcinogenic risks for Pb consumption in the samples were not demonstrated in the present study. THQ value for Cd was recorded highest in raw Blackchin Tilapia and Nile Tilapia present also from Los Baños with scores 1.63 and 1.30 respectively. The scores were relatively higher than the recommended level of exposure (THQ  $\geq$  1.00). Thus, daily exposure at this level could likely cause an adverse health effect. Average THQ for Chromium, on the other hand, scored highest again in Los Baños for both species. However, all values did not exceed the recommended threshold of 1.0 thus the risk for the non-carcinogenic effect of Cr is low. Moreover, the THQ values for all concerned heavy metals were comparatively higher in females than in males. This is probably due to the difference in the average weight.

## DISCUSSION

There were several studies conducted to assess the heavy metal contamination in aquatic animals collected from different sampling zones in Laguna Lake. In the study conducted by de la Cruz et al. (2016), Asian clams collected from different sampling zones in Laguna Lake showed detectable levels of Pb in soft tissue samples. Similar to the present study, the [Pb] showed higher concentrations detected in samples obtained from sites adjacent to the urban areas, Los Baños and Calamba, compared to samples collected from collection sites facing the rural towns of Bay, Victoria, and Kalayaan. Growing cities surfaced with factories and industrial parks that high concentrations of heavy metals dumped in the lake have become a major water quality problem.<sup>18</sup>

The concentration level in the head part of the fish could be attributed to the fact that water always passes through the mouth and gills when filtered. Bervoets et al. (2001) reported that more concentration of heavy metals accumulates in the gill than the liver followed by muscle and gut region for short time exposure.<sup>19</sup> Varying levels of metal bioaccumulation in different parts of fish are attributed to the differences in their physiological function. Fish regulate metal ions through excretion via kidney and gills, however, such capacity of tissue is directly related to the total amount of metals' accumulation in those specific tissues.<sup>20</sup>

Several studies have indicated an increase in heavy metal concentration in foods using different cooking methods. According to the results of study conducted by Gheisari et al. (2016) on the effect of different cooking methods on lead and cadmium contents of shrimp and lobster, the concentrations of lead (Pb) increased in fried samples showing significant differences when compared with steamed and boiled shrimp and boiled lobster ( $P < 0.05$ ).<sup>21</sup> Moreover, investigations of Ersoy et al. (2006) on lead (Pb) and cadmium (Cd) concentrations in sea bass under four treatments also showed that lead (Pb) concentrations in the baked and microwaved fish were significantly decreased.<sup>22</sup> Although the reasons behind such differences are not quite clear, many factors such as the initial concentration of heavy metals in the meat, aquatic species, can affect the reduction or increase of these elements in the flesh as the result of cooking processes.<sup>21</sup>

Results of the risk assessment for the present study show that cadmium is of greater concern in terms of possible adverse health effects associated with heavy metal bioaccumulation. However, the presence of other heavy metals in fish species should not be deserted. Although there are limitations in establishing the risk for multiple exposures, it must be noted that various chemical exposure can also lead to increased health risk.

Studies suggest that excess levels of this chemical may pose serious health problems in humans. Lead can cause effects on the blood, as well as the nervous, immune, renal and cardiovascular systems. Early childhood and prenatal exposures are associated with slowed cognitive development, learning deficits and other effects. Exposure to high amounts of lead can cause gastrointestinal symptoms, severely damage the brain and kidneys, and may cause reproductive effects. Large doses of some lead compounds have caused cancer in lab animals.<sup>23</sup> On the other hand, the kidney is the critical target organ in humans for cadmium toxicity. Cadmium accumulates primarily in the kidneys, and its biological half-life in humans is 10–35 years. This accumulation may lead to renal tubular dysfunction and can lead to disturbances in calcium metabolism and the formation of kidney stones. Softening of the bones and osteoporosis may occur in those exposed through living or working in cadmium-contaminated areas.<sup>24</sup> According to USEPA, ingestion of high amounts of chromium causes gastrointestinal effects in humans and animals, including abdominal pain, vomiting, and hemorrhage.<sup>25</sup>

## CONCLUSION

Results of the study showed that the average [Pb], [Cd] and [Cr] were all recorded highest from fish samples obtained from Los Baños while lowest in Bay. Among the fish parts, the head part accumulated the highest concentration of all heavy metal followed by muscle and stomach. The heavy metal contents of all samples were significantly lower in boiled samples compared to raw samples. The difference ranged from 50% to 75% for all heavy metals in both species.

Estimated Daily Intake (EDI) of Pb from raw and boiled Blackchin Tilapia and Nile Tilapia, exceeded the maximum permissible limit except in boiled Blackchin Tilapia in Bay. Higher EDI of Cd was also recorded compared to the maximum permissible limit in most of the samples. Meanwhile, all computed EDI of Cr did not exceed its maximum permissible limit.

Adverse health effects associated with Nile Tilapia and Blackchin Tilapia consumption were demonstrated in this study. Among the three metals, THQ value for Cd was recorded highest in raw Blackchin Tilapia and Nile Tilapia collected from Los Baños. The scores for Cd were relatively higher than the recommended level of exposure ( $THQ \geq 1.00$ ) which indicates risk for non-carcinogenic health effects. Total hazard Quotient for Pb and Cr did not exceed the recommended value ( $THQ < 1.0$ ). Nevertheless, there is still a possibility of an increase in health risk if there is prolonged exposure to multiple chemicals such as heavy metals.

## Statement of Authorship

All authors participated in data collection and analysis, and approved the final version submitted.

## Author Disclosure

All authors declare no conflicts of interest.

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