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Metal Bioaccumulation by Carp and Catfish Cultured in Lake Chapala, and Weekly Intake Assessment

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Abstract: Aquaculture offers great potential for fish production in Lake Chapala, but reports of heavy metal contamination in fish have identified a main concern for this activity. In the present study, cultures of the species *Cyprinus carpio* and *Ictalurus punctatus* were grown in a net cage in Lake Chapala. The patterns of heavy metal accumulation (Cu, Zn, Cd, Hg, Pb, As) in muscle and liver were monitored in order to evaluate the level of metal incorporation in the fish. Estimates of weekly metal intake (EWI) were made based on the results of the concentrations in edible parts of fish of commercial size. The patterns of metal bioaccumulation between tissues and species showed that liver had a higher concentrating capacity for Zn, Cu, Cd, and Pb. In contrast, similar concentrations of Hg and As were found in the liver and muscle tissue. According to the EWI estimates, the heavy metals in these cultured fish do not represent a risk for human consumption.

Keywords: heavy metals; bioaccumulation; Lake Chapala; carp; catfish; PTWI

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Citation: Alvarado, C.; Cortez-Valladolid, D.M.; Herrera-López, E.J.; Godínez, X.; Ramírez, J.M. Metal Bioaccumulation by Carp and Catfish Cultured in Lake Chapala, and Weekly Intake Assessment. *Appl. Sci.* **2021**, *11*, 6087. https://doi.org/10.3390/app11136087

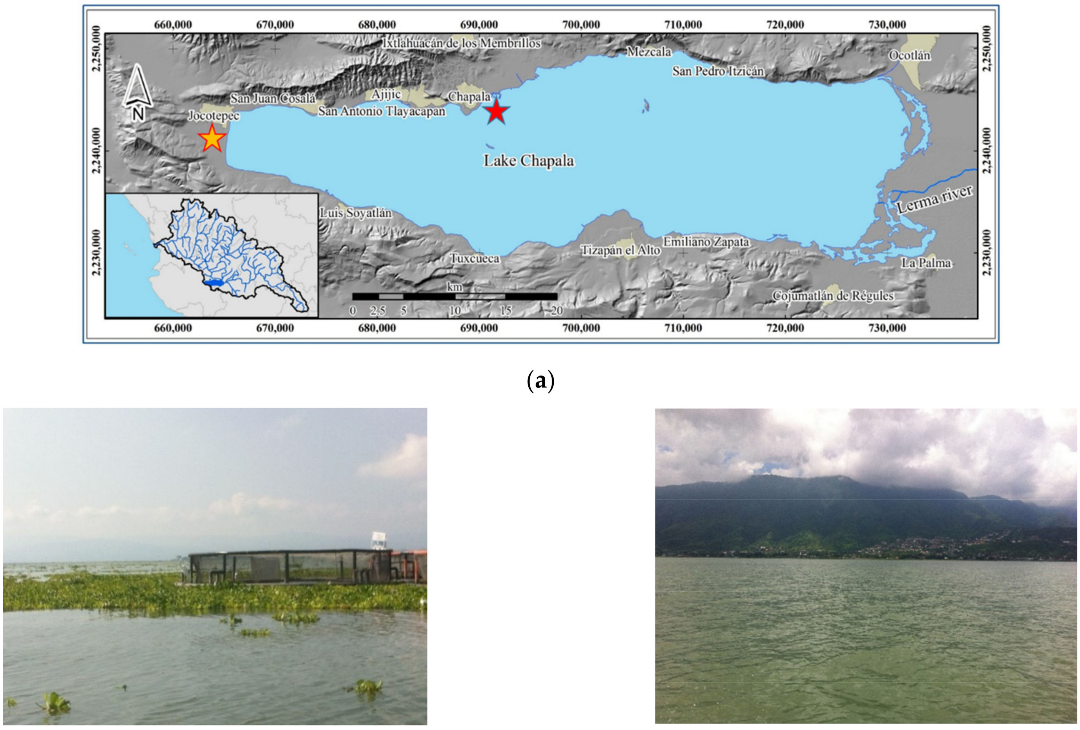
Academic Editor: Elida Nora Ferri

Received: 28 April 2021
Accepted: 23 June 2021
Published: 30 June 2021

1. Introduction

Lake Chapala is the largest freshwater lake in Mexico, with a total surface area of 114,659 ha [1]. A shallow, alkaline body of water, it has a mean depth of less than 7 m. Lake Chapala belongs to the Lerma-Chapala Basin. Its main tributary input comes from the Lerma River, which supplies about 80% of its waters (Figure 1). This river is also the main source of anthropogenic pollution, including heavy metals, because ~3500 industries pour their treated wastewaters into it [2,3]. Sediments from Lake Chapala are rich in heavy metals, and previous studies have shown that lead (Pb) and cadmium (Cd) are present in the exchangeable fraction of water, and possibly available for absorption by living organisms, including fish [4]. The sediments, however, have a metal adsorption–desorption dynamic that is strongly influenced by pH and marked seasonal fluctuations in water levels, so low concentrations of metals in the water have also been reported [5,6].

For humans, consuming fish is an excellent source of proteins and n-3 polyunsaturated fatty acids, but concern has arisen over the potential danger of heavy metals [7]. Human diets are a significant source of exposure to heavy metals that carry a toxicological risk because they cannot be degraded by biological processes. Due to this potential for harm, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established a Provisional Tolerable Weekly Intake (PTWI) for metals that estimates the amount of a given contaminant that can be assimilated weekly per unit of body weight (bw) over a lifetime, without constituting an appreciable health risk [8].



(**a**) (**b**) (**c**)

Figure 1. Location of the experimental cage in Lake Chapala. (**a**) Map of Lake Chapala showing with the red star the experimental cage and the yellow star the control group. (**b**) Experimental cage. (**c**) General view of Lake Chapala environment.

Contaminated fish are the principal source of heavy metals such as mercury, lead, and arsenic for humans. Previous reports in fish from Mexican water reservoirs have shown borderline-to-high quantities of heavy metals, making it essential to maintain constant monitoring of their intake through fish consumption in the diet [9–12].

The aim of the present study was to evaluate the bioaccumulation of metals (Zn, Cu, Cd, Hg, As, Pb) along time in fish cultured in a natural environment to evaluate the risk of dietary intake of metals in humans.

2. Materials and Methods

2.1. Study Design and Sampling

About one thousand catfish fingerlings (*Ictalurus punctatus*) aged 15 weeks (average weight 4.80 ± 2.96 g and length 8.51 ± 2.04 cm), and two hundred adult carps (*Cyprinus carpio*) aged 30 weeks (average weight 30.04 ± 15.55 g, and length 11.08 ± 4.69 cm) were cultured in the same floating net cage in Lake Chapala, Jalisco, Mexico (coordinates 20°17′2.9′′ N, −103°10′27.2′′ W) (Figure 1). These fish were called the experimental group. The cage measured 6 m in diameter and 2 m deep, with a surface area of 100.13 m^2 and volume of 56 m^3. The mesh size was 1 inch. It was placed 3 m above the bottom of the lake. During the fingerling age period, the catfish were kept in a nursery cage within the larger cage, and after they remained in it for 3 months, fish were released. In order to detect any factor that might affect metal bioaccumulation, a control group of fish with the same characteristics was cultured in an earth pond in Jocotepec, Jalisco, a lakeside community (Figure 1).

The fish were fed Winfish-Zeigler 3506 (3.5 mm) until they reached a weight of 50 g (6 months), when this was replaced with Winfish-Ziegler 2505 (5.5 mm). Culture performance was monitored monthly in terms of biometrics, morbidity, and mortality. The properties of the lake, monitored and recorded weekly, were pH, temperature, and dissolved oxygen. Table 1 shows the metals concentration of the feedstuffs.

Table 1. Metal concentration of fish feedstuffs and the maximum content allowed.

Metal	Winfish-Ziegler 3506 (3.5 mm)	Winfish-Ziegler 2505 (5.5 mm)	Maximum Content [13]
Cu (mg/kg)	21.77 ± 2.45	52.83 ± 1.58	-
Zn (mg/kg)	216.50 ± 10.89	94.86 ± 2.53	-
Cd (µg/kg)	0.36 ± 0.34	0.08 ± 0.01	1000
As (µg/kg)	0.90 ± 0.28	0.43 ± 0.02	6000
Pb (µg/kg)	1.24 ± 0.05	0.81 ± 0.08	5000
Hg (µg/kg)	17.89 ± 3.16	13.17 ± 0.52	500

The collection of fish samples involved taking 3–5 individuals from each species and for each experimental group at intervals of 6–7 weeks of exposure, concretely on days 0, 38, 81, 123, 179, 241, 298, 369, 424, and 473. For sampling, the fish were transferred to containers with 0.2 g/L of MS 222 anesthetic (Sigma Aldrich). When dead, the fish were placed into new plastic bags and transported to the laboratory in a container to maintain the chain of command. The length and weight of each fish and its tissues were recorded before dissection. Liver and muscle tissue were removed, weighed, and homogenized individually, except for catfish, at the three first sampling stages where the fish were very small, and the same tissues from different fish were placed in the same bag. Samples were frozen at −20 °C for further analysis.

2.2. Analysis of Metals

The concentrations of copper (Cu), zinc (Zn), cadmium (Cd), lead (Pb), mercury (Hg), and arsenic (As) were analyzed in the liver and muscles, as well as in the fish food. The samples of fish tissues and fish food were treated by microwave digestion with nitric acid, according to the 5BI-8 sample preparation note. Briefly, 1.00 ± 0.05 g of each homogenized sample was weighed in microwave vessels and HNO_3 was added. The vessels were placed in a CEM Marx microwave and heated at 1200 W, 200 psi, and 210 °C for 10 min, then allowed to cool at room temperature until the digestion time was completed. The samples were then gauged to 50 mL with deionized water.

All samples were analyzed in duplicate. Cu and Zn concentrations were measured by inductively coupled plasma optical emission spectroscopy (ICP-OES) in a Perkin Elmer Optima 8300DV (Shelton, CT) using internal method INS-SM/US-71, based on EPA method 6010B. For Cd, Pb, Hg, and As, internal method INS-SM/US-220 was followed using inductively coupled plasma mass spectrometry (ICP-MS) in a Perkin Elmer ELAN 9000 (Shelton, CT, USA).

Standard Perkin Elmer solutions (N9300174) were used to prepare the calibration curves, which presented $R2 > 0.998$. The recovery percentages performed by spiked samples of the fish ranged from 90% to 110%. Precision was measured by evaluating a Perkin Elmer N9300211 solution. The coefficients of variation did not exceed 9%. DORM-4 (fish protein) reference material from Canada's National Research Council (CNRC, Ottawa, Canada) and FAPAS T07213QC (crab meat) reference material from The Food and Environmental Research Agency (FAPAS, Sand Hutton, UK) were digested and analyzed in triplicate for quality control. Table 2 shows the recoveries of heavy metals and the corresponding certified values for the reference materials, according to the method used.

Table 2. Certified concentration and measured values for reference material DORM-4 and FAPAS T07213QC by method.

Element	Reference Material	Certified Conc.	Units	Measured Concentration	Detection Limit	Method
Hg	DORM-4	410	μg/kg	385	1.9	ICP-MS
Hg	FAPAS T07213QC	93.5	μg/kg	76.7	1.9	ICP-MS
Cu	DORM-4	15.9	mg/kg	14.8	0.32	ICP-OES
Zn	DORM-4	52.2	mg/kg	57.37	1.16	ICP-OES
Pb	DORM-4	416	μg/kg	423	0.287	ICP-MS
Pb	FAPAS T07213QC	50.1	μg/kg	47.0	0.287	ICP-MS
Cd	DORM-4	306	μg/kg	301	1.3	ICP-MS
Cd	FAPAS T07213QC	5.53	μg/kg	5.78	1.3	ICP-MS
As	DORM-4	6800	μg/kg	7158	1.8	ICP-MS
As	FAPAS T07213QC	13.9	μg/kg	14.0	1.8	ICP-MS

2.3. Estimated Weekly Intake

The estimated weekly intake (EWI) of essential (Cu, Zn) and non-essential metals (Cd, Pb, As, Hg) through consumption of the cultured catfish and carp were calculated, assuming a weekly consumption of 200 g for Latin American populations [14] and an average body weight of 70 kg for Mexican people [15]. Calculations were performed by applying the mean and maximum concentrations for each metal analyzed in the study in the following equation:

$$\textbf{EWI} = \frac{\textbf{Metal Concentration} \times \textbf{Fish intake}\ (0.2\ \textbf{kg})}{\textbf{Body weight}\ (70\ \textbf{kg})}$$

Results were compared as the percentage of contribution to their respective Provisional Tolerable Weekly Intake (PTWI) value established by JECFA [8]:

$$\%\ \textbf{PTWI} = \frac{\textbf{EWI}}{\textbf{PTWI}} \times 100$$

2.4. Data Processing

The data from the catfish and carp were stored in Excel® software. Data were ordered according to the metals Hg, Cu, Zn, Pb, Cd, and As evaluated in the liver and muscle of the fish species.

The liver concentration factor (LCF) was obtained by dividing the mean concentration of every metal in the liver over the mean concentration of the metal in muscle, for every time in the experimental group. Diverse trendlines—linear, exponential, and logarithmic, among others—were evaluated to properly fit the catfish and carp data. The R2 coefficient and visual inspection were used as criteria to select the most appropriate function for representing the shape of the fitted data. In a second approach, the data were analyzed, and the mathematical expressions selected for the fitted data were imported into Matlab® 2015 software, which generated numerical vectors for each data category as a function of sampling time to represent the concentrations of metals that had accumulated in the organs examined. Our experimental data were then plotted versus the interpolated curves generated by the mathematical equations obtained during the fitting procedure.

2.5. Statistical Analyses

Means and standard deviations of every point in time by fish were then determined for every data vector, experimental versus control. F-tests run in STATGRAPHICS® 2018 software were used to determine significant differences between the standard deviations of the two samples at a confidence level of 95.0%.

3. Results

3.1. Pattern of Metal Bioaccumulation

3.1.1. Essential Metals: Cu and Zn

Table 3 shows the range of metal concentrations in catfish and carp obtained for every metal and the ranges of LCF for fish cultured in Lake Chapala, named the experimental group. Figures 2 and 3 show the pattern of nutritive metal bioaccumulation in muscle and liver of the cultured carp and catfish in experimental and control groups. Table 4 shows the differences between experimental and control groups, for every metal, species, and tissue. Cu and Zn did not show statistical differences between groups.

Table 3. Range of metal concentrations in muscle and liver of cultured fish, and range of liver concentration factor (LCF) by fish species in the experimental group along time.

Metal	Carp (*Cyprinus carpio*)			Catfish (*Ictalurus punctatus*)		
	Muscle	**Liver**	**LCF**	**Muscle**	**Liver**	**LCF**
Cu (mg/kg)	0.36–2.39	6.44–47.81	9–37	0.56–3.83	1.48–5.84	1–9
Zn (mg/kg)	8.84–16.05	38.44–265.00	2–28	5.66–12.64	17.39–28.79	2–4
Cd (µg/kg)	<1.3–3.67	13.25–472.40	8–220	<1.3–2.14	5.39–51.56	1–42
Pb (µg/kg)	3.25–17.44	9.70–76.38	0.6–23	3.51–15.23	2.87–23.90	0.5–3
As (µg/kg)	43.73–192.78	38.35–126.08	0.3–3	10.57–126.40	11.47–87.34	0.3–3
Hg (µg/kg)	<1.9–5.88	<1.9–18.59	0.3–5	<1.9–34.56	<1.9–24.60	0.5–1

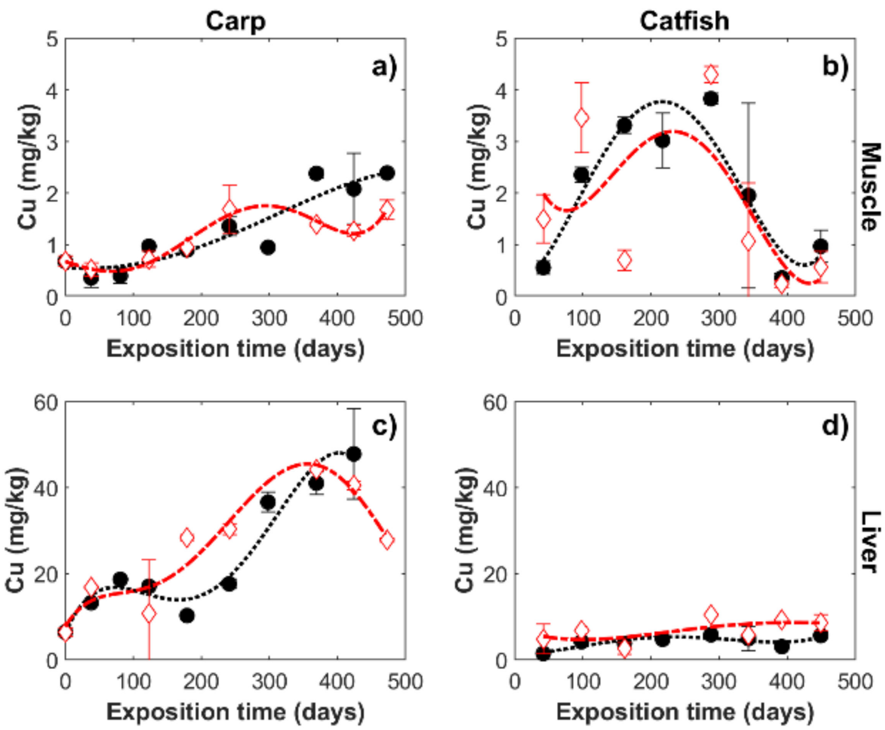


Figure 2. Cu accumulation in the experimental (•) and control groups (◇), in front of (**a**) muscle in carp, (**b**) muscle in catfish, (**c**) liver in carp, and (**d**) liver in catfish.

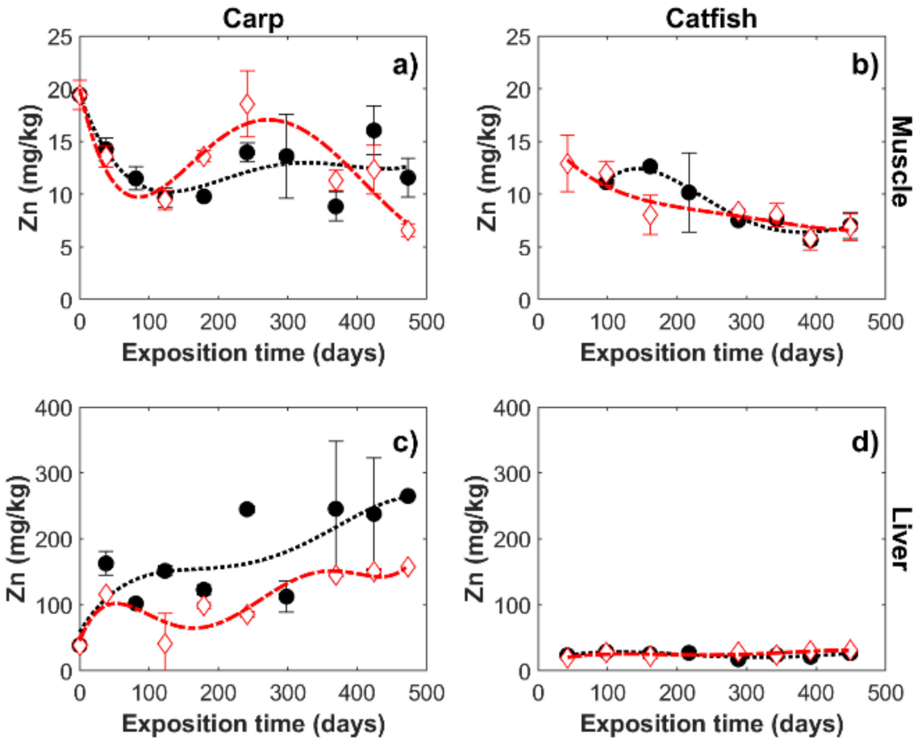


Figure 3. Zn accumulation in the experimental (•) and control groups (◇), in front of (**a**) muscle in carp, (**b**) muscle in catfish, (**c**) liver in carp, and (**d**) liver in catfish.

Table 4. F-test to compare the variances of the experimental and control groups for carp and catfish, in muscle and liver.

Metal	Fish	Muscle		Liver	
		F	*p*-Value	F	*p*-Value
Cu	Carp	3.017	0.168	1.213	0.820
	Catfish	0.747	0.732	0.317	0.188
Zn	Carp	0.722	0.679	2.797	0.198
	Catfish	1.661	0.591	0.711	0.689
Cd	Carp	0.361	0.240	156.912	0.000 *
	Catfish	1.038	0.965	0.301	0.213
Pb	Carp	0.582	0.528	0.368	0.249
	Catfish	0.008	0.000 *	0.605	0.557
As	Carp	0.793	0.786	0.253	0.158
	Catfish	2.130	0.380	1.193	0.851
Hg	Carp	1.247	0.796	0.429	0.433
	Catfish	1.112	0.921	2.240	0.454

* Significant differences between the control and experimental group.

- Copper

Figure 2a,b show the Cu accumulation patterns in the muscle of both species and in both groups. A similar concentration range was observed in the muscle of both species (Table 3), but the pattern of accumulation showed large differences, as the concentration in the carp showed a steady increase over time, while in the catfish, a curve with a maximum on day 288 was observed. This behavior in carp muscle was observed in both groups, experimental and control.

Figure 2c,d show the accumulation patterns of Cu in the fish livers. Both species showed higher concentrations in the liver, but the carp had levels 22 times higher than in the muscle tissue (Table 3), 6.44–47.81 mg/kg in liver versus 0.36–2.39 mg/kg in muscle. The catfish liver, in contrast, showed an accumulation only three times as large as that of the muscle tissue (1.48–5.84 mg/kg in liver versus 0.56–3.83 mg/kg in muscle). The carp liver (6.44–47.81 mg/kg) also had a concentration 10 times higher than the catfish liver of the experimental group (1.48–5.84 mg/kg).

- Zinc

Figure 3a,b show the Zn accumulation pattern in the muscles of both species. The range of concentrations for experimental groups remained in a range between 5.66 and 16.05 mg/kg for both species, and the general pattern of concentration described a descending trend. The experimental and control groups showed similar patterns of bioaccumulation.

Zn accumulation in the liver of both species was higher than in the muscle (Figure 3c,d). Zn concentrations in the liver began at similar levels in the two species, but a constant increase was seen in the carp. At the end of the experiment, the carp liver had 10 times more Zn than the catfish liver (265.00 versus 28.79 mg/kg) in experimental groups.

3.1.2. Non-Essential Metals: Cd, As, Pb, and Hg

- Cadmium

Figure 4a,b show the Cd accumulation pattern in the muscle of the catfish and carp. Muscle concentrations in both species were in the same range (1.3–3.67 µg/kg). The accumulation pattern in the carp showed a slight rise over time, but in the catfish, it was rather flat. Only carp showed significant differences in liver between experimental and control groups (Table 4).

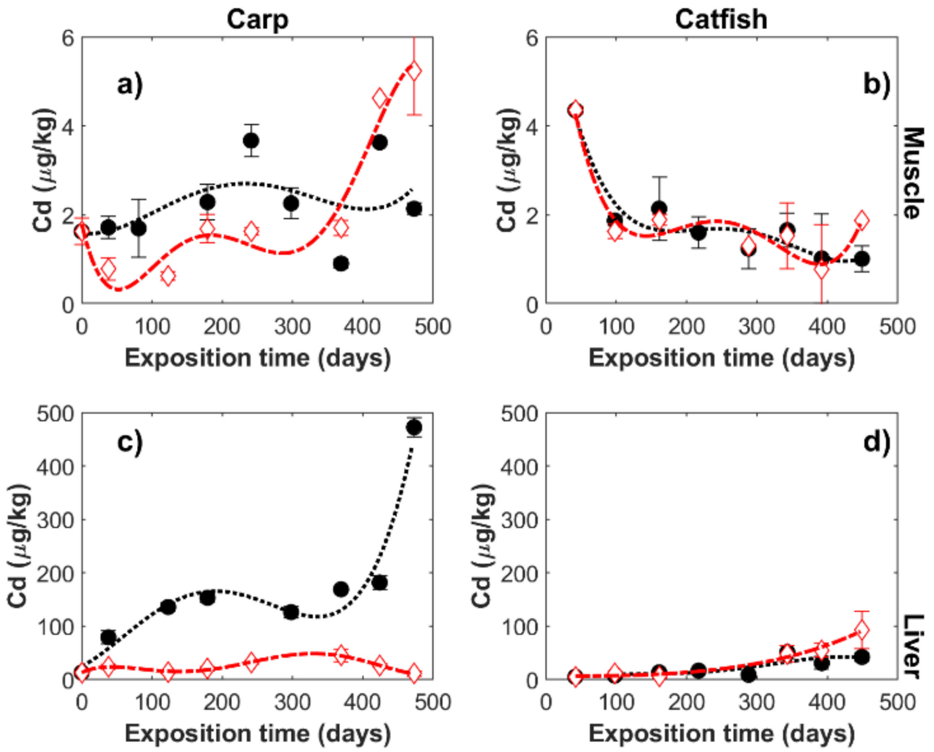


Figure 4. Cd accumulation in the experimental (•) and control groups (◇), in front of (**a**) muscle in carp, (**b**) muscle in catfish, (**c**) liver in carp, and (**d**) liver in catfish.

On average, Cd concentrations in carp were 78 times higher in the liver than muscle and 17 times higher in the case of catfish in the experimental group, revealing the concentrating function of liver in both species (Figure 4c,d). The Cd concentration in the carp liver was twice as high as that of the catfish at the beginning of the experiment, but by the end, it was almost 10 times higher (472.40 versus 51.56 µg/kg) for the carps cultured in Lake Chapala only. The general pattern of metal accumulation had an upward trend in the liver of the experimental fish that was more prominent in the carp. The carp cultured in Lake Chapala had a significantly high concentration in liver with respect to the carp in the earth pond.

- Lead

Figure 5a,b show the Pb accumulation pattern in the muscle of the fish. The concentration ranges were very similar between both species and organs over time (Table 3). Only in the catfish muscle did the control group show a significantly higher concentration at the beginning of the experiment (Table 4).

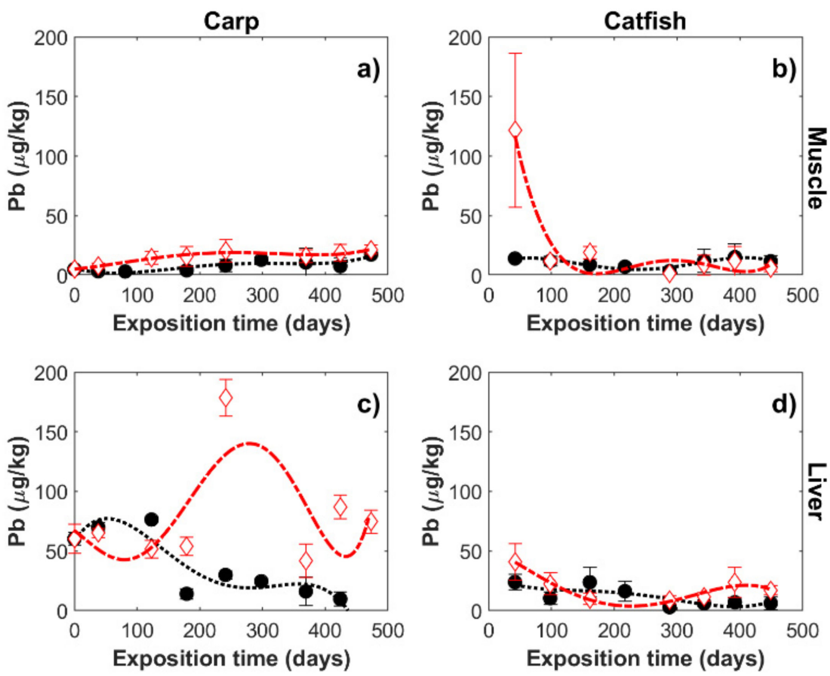


Figure 5. Pb accumulation in the experimental (•) and control groups (◇), in front of (**a**) muscle in carp, (**b**) muscle in catfish, (**c**) liver in carp, and (**d**) liver in catfish.

In the catfish liver, the Pb concentrations were on average 1.2 times higher than in the muscle. LCF between 11 to 23 was observed in carp liver, which gradually decreased after day 123 (Figure 5c,d).

- Arsenic

Figure 6a,b show the As accumulation pattern in the muscles of the catfish and carp. In this case, a downward trend of bioaccumulation was observed in the muscle of both species. Concentrations were slightly higher in the carp than the catfish, but within the same range (Table 3). As did not show statistical differences between groups (Table 4). The carps of the experimental group had on average 15 µg/kg of As higher than the control group, but after 298 days of exposition, they reversed the trend. No liver concentration effect was observed for As in either species, as the concentrations were slightly lower than in the muscle throughout the study period (Table 2; Figure 6c,d). The catfish and carp livers had ranges of 11.47–87.34 and 38.35–126.08 µg/kg, respectively. The general trend in the carp liver showed a soft upward shape, but the trend in the catfish was flat both in the experimental and control groups (Figure 6d).

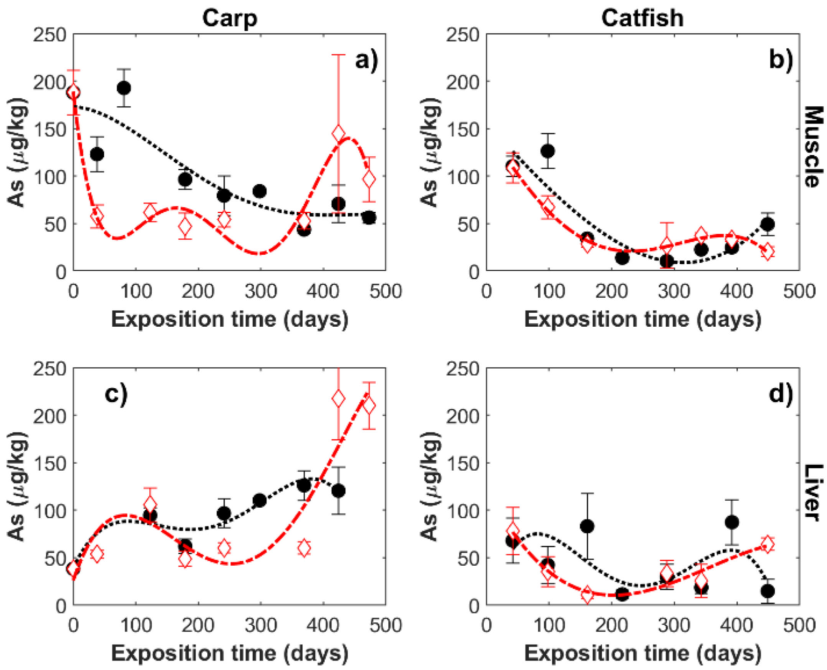


Figure 6. As accumulation in the experimental (•) and control groups (◇), in front of (**a**) muscle in carp, (**b**) muscle in catfish, (**c**) liver in carp, and (**d**) liver in catfish.

- Mercury

Figure 7 shows the Hg bioaccumulation in the muscle and liver of the two species. Hg did not show statistical differences between groups (Table 4). No liver accumulation effect was observed in catfish, and soft LCF was observed in carp (0.3 to 5). In fact, in many samples, concentrations were below the method's detection limit (1.9 μg/kg). Concentration ranges were similar between the tissues (Table 2), and lower in the carp (<1.9–18.59 μg/kg) than the catfish (<1.9–34.56 μg/kg). A slightly upward trend of bioaccumulation was observed in both species in muscle.

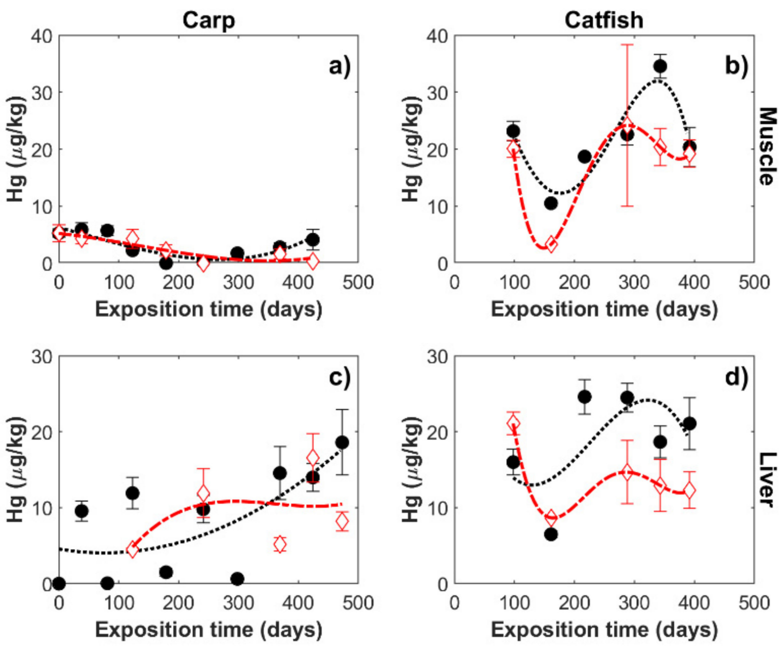


Figure 7. Hg accumulation in the experimental (•) and control groups (◇), in front of (**a**) muscle in carp, (**b**) muscle in catfish, (**c**) liver in carp, and (**d**) liver in catfish.

3.2. Estimated Weekly Intake

The means of the metal concentrations obtained from the muscle tissue of commercial-sized fish in experimental and control groups are summarized in Table 5. This information was used to calculate the EWI and the percentages of contribution to the PTWI of each metal (Table 5).

Table 5. Mean concentration (ww) of metals in fish muscle at consumption size in experimental and control groups. Estimated weekly intake (EWI) and contribution to Provisional Tolerable Weekly Intake (%PTWI) through consumption of cultured fish.

Metal	Fish	Control Group			Experimental Group			Reference PTWI †
		Conc.	EWI *	% PTWI	Conc.	EWI *	% PTWI	
Cu (mg/kg)	Carp	1.45 ± 0.21	0.004	0.12	2.28 ± 0.18	0.007	0.19	0.5
	Catfish	0.63 ± 0.42	0.002	0.05	1.09 ± 0.80	0.003	0.09	
Zn (mg/kg)	Carp	10.08 ± 3.07	0.029	0.41	12.16 ± 3.64	0.035	0.50	1.0
	Catfish	6.93 ± 1.11	0.020	0.28	6.78 ± 1.02	0.019	0.28	
Cd (μg/kg)	Carp	3.86 ± 1.88	0.011	0.18	2.23 ± 1.36	0.006	0.11	6.0
	Catfish	1.40 ± 0.56	0.004	0.07	1.23 ± 0.37	0.004	0.06	
Pb (μg/kg)	Carp	18.85 ± 2.85	0.054	0.22	11.97 ± 4.98	0.034	0.14	25
	Catfish	8.97 ± 3.02	0.026	0.10	13.03 ± 1.93	0.037	0.15	
As (μg/kg)	Carp	98.05 ± 45.76	0.280	1.87	57.01 ± 13.55	0.163	1.09	15
	Catfish	30.54 ± 8.74	0.087	0.58	32.39 ± 14.74	0.093	0.62	
Hg (μg/kg)	Carp	0.98 ± 0.91	0.003	0.18	3.44 ± 0.93	0.010	0.61	1.6
	Catfish	19.78 ± 0.82	0.057	3.53	27.46 ± 10.04	0.078	4.90	

* Estimated weekly intake for a 70 kg person (μg/kg bw per week). † Provisional Tolerable Weekly Intake (μg/kg bw per week) [8].

The cultured catfish and carp showed low concentrations of Cu and Zn, and very low EWI values, with maximums of 0.007 and 0.068 μg/kg of bw respectively, as shown in Table 5.

The estimates of weekly intakes of non-essential metals in the experimental group were below the respective PTWI for both species. The contributions of Cd, Pb, and As represented less than 1.5% of the total PTWI, while the values for Hg ranged from 0.61% to 4.90% (Table 5).

4. Discussion

The between-tissue and between-species analyses of the metal bioaccumulation patterns showed a higher concentrating capacity of the liver for Zn, Cu, Cd, and Pb compared to the muscle tissue. In contrast, the liver showed similar or lower concentrations of Hg and As than the muscle. These findings of the liver's higher capacity for concentrating Zn, Cu, and Cd agree with previous reports on the sequestrating–detoxifying function of the liver, which involves the action of the protein (Cd, Zn)-metallothionein [16–18]. The liver's high capacity for metal accumulation has been documented extensively in cyprinids for these three metals [17,19] and in several families of catfish [20–23].

The comparison of the liver's accumulation capacity for Cu and Zn between the two species analyzed showed that for the carp, it was 10 times greater than that of the catfish. The findings were observed in both the experimental and the control groups, and the results disagree with previous reports, since a higher capacity in catfish liver compared to *Cyprinidae* species has been reported in wild fish in natural environments [24,25]. The explanation of the higher concentration obtained in the carp in our study could be attributable to two factors. Studies of fish in the same aquatic environment have reported feeding behavior and trophic position as the main sources of metals [26,27]. In our work, however, both species cultured in Lake Chapala were fed the same commercial food in the same cage, so they had only limited access to food from the environment. As is observed in the results section, metal concentrations in the commercial food were low, so under conditions in which the diet of the fish is controlled, their natural feeding habits and their effect on metal accumulation will not be reflected.

On the other hand, diverse studies have reported that the age of the fish correlates positively with metal accumulation [24,28]. In the present work, the age of the catfish (98–545 days) was lower than that of the carp (240–713 days). We have one moment at the same age of fish that could be comparable, at 538 days of carp (288 days of exposure) and the catfish aged 545 days (449 days of exposure), the Zn and Cd concentration factors (LCF) were closer. Only Cu maintained a large between-species difference, which was observed from the early stages of the accumulation pattern. We conclude, therefore, that at the same age, and under similar dietary and environmental conditions, the livers of the catfish and carp maintained similar concentrating capacities for Zn and Cd, but not for Cu. The catfish liver showed a different bioaccumulation pattern for Cu than the carp, with the latter generating an accumulation factor lower than the former.

The Cd pattern of accumulation in liver showed a clear difference between experimental and control groups only in the carp case, as is observed in Figure 4c. The Cd concentration at the end of the exposition time was 472.40 μg/kg in the experimental group versus only 23.42 μg/kg for the control. The effect is attributable to the lake, although the Cd concentration in water was acceptable (0.01 ± 0.00 μg/L) [6]. The Cd concentration in liver was far from toxic limits according to previous studies in sub-lethal exposition, where the liver Cd concentrations were between 39,300.00 and 46,100.00 μg/L [16,17]. However, it is advisable to continue monitoring the fish in the lake to determine the maximum level reached by Cd in the liver. The concentrations of As, Hg, and Pb found in the tissues analyzed in this study tended to be lower than those reported previously for the same fish species in the wild [9,12,24,25,29]. With respect to aquaculture fish, our literature review only identified reports on carp species. The concentrations of As and Pb found in the muscle in those studies [30,31] were similar to our results. In another finding, the Hg concentrations in the muscle of carp in the present study were 400-fold less than those reported in [31]. The difference could be attributed to the fact that that work was conducted in wild carp, with free contact with sediments and contaminated sources of food. Therefore, maintaining control of the feed administered reduced the possibility of the fish accumulating heavy metals.

The liver from both species showed a 1.5–4-fold capacity for accumulating Pb compared to the muscle, capacities much lower than those of Cu, Zn, and Cd (Table 2). The concentrations of As and Hg, however, were either similar or lower in the liver than in the muscle in both species. Here, our results are consistent with the patterns of Pb, As, and Hg accumulation cited in previous studies of catfish [22–24], and carp tissues [24,25,30].

The main uptake pathway for Pb is through the water [25]. Thus, the concentration of Pb in the water is decisive for bioaccumulation in fish. The Pb concentrations in the water samples taken from Lake Chapala during the experiment (0.33–0.37 μg/L) [6] were below Mexican standards (10.00 μg/L) [32]. This could account for the low Pb concentrations found in the tissues of both species.

Studies of the mechanism of As and Hg accumulation in fish have been conducted at different trophic levels. The form of the chemical is another key factor in fish metal uptake. V (arsenate) and methylmercury (MeHg), respectively, are the bioavailable forms of As and Hg [33,34]. Food is the main pathway of metal intake by fish, but while the biomagnification phenomenon has been observed for Hg through the tropic chain with higher concentrations in predator species, As has not been associated with trophic position [33,35,36]. The heavy metals in the fish commercial food were always lower than international standards [13]. This explains the low As and Hg concentrations found in the fish in our study, since food was provided as in standard aquaculture systems with limited access to natural food sources.

Another factor that plays a role in the uptake of metals in aquatic organisms is pH. Lake Chapala maintained a pH of 8.6–9.5 during our experiment [6]. This alkaline pH promotes the aggregation of metals into particles that settle, thus reducing their dissolution in the liquid phase [5]. This explains the low metal concentration found in the water of Lake Chapala despite the high concentration of its sediments [6].

The data in Table 5 indicate the EWI levels for the fish from Lake Chapala in the experimental and control groups, and all of them were below 1% of the PTWI. Regarding the intake of Cu and Zn by fish consumption as essential metals, the values obtained are part of normal human dietary requirements.

The risk of consuming fish revolves around their content of non-essential heavy metals. Table 6 presents the comparison of the EWI and % PTWI estimated during the present study from the fish cultivated in Lake Chapala, with other reports of cultured and wild freshwater fish.

Table 6 shows that the EWI and % PTWI values for Cd in the present work were similar (0.11%), although lower than the unique report of cultured carp (0.35%) [30], as well as others reports of wild fish from lakes [30,37]. On the other hand, some previous works showed EWI up to two orders of magnitude higher than our work (0.395 and 0.743), and PTWI higher than 5%, which is indicative of caution [12,38].

The EWI values for lead in the present work were one or two orders of magnitude lower than previous reports, as shown in Table 5. Nevarez et al. [12] showed the highest EWI values for lead in native catfish (7.307 μg/kg of bw) living in a dam environment. According to the authors, the causes were a natural, local source of Pb, runoff from rain, and residue from extractive mining. It is interesting to notice that Nevarez et al. [12] and Alipour and Banagar [38] reported the highest % PTWI in two metals, Cd and Pb.

The EWI for As obtained in the present work for carp (0.163 μg/kg of bw) was lower, although it resulted in the same range as those reported by Alam et al. [30] for native and cultured carp (0.271 and 0.511 μg/kg of bw, respectively). The water concentrations during experiments were slightly higher in Lake Chapala (9.22–11.0 μg/L) [6] compared to the values reported by Alam et al. [39] (0.72 to 3.1 μg/L). Regarding EWI for catfish, the obtained 0.093 μg/kg of bw agrees with Nevarez et al. [9], who reported 0.189 μg/kg of bw in the same type of fish. The As water concentration reported by Nevarez et al. [9] (1.34–5.65 μg/L) was in a similar range as that in the present study. The % PTWI was less than 5% in the previous reports and agrees with their water and fish As concentration.

Mercury is the principal toxic metal of concern in terms of consuming fish and seafood, which constitute the primary sources of this metal [40]. In the present study, Hg had 0.61% and 4.9% of the PTWI in carp and catfish, respectively. Based on the mean mercury concentrations reported by Trasande et al. [41], Stong et al. [10], and Torres et al. [11] for native carp from Lake Chapala, a decrease in the concentration is noted over time that results in a lower weekly intake from native fish that represented a contribution to the PTWI of 155.36% in 2010 and 40.89% in 2014. Clearly, these figures are much higher than the results of our study, emphasizing the decrease in metal accumulation that is evident in fish cultured in floating cages. In another study, Nevarez et al. [12] reported an EWI of a dam catfish (0.124 μg/kg of bw), while Łuczyńska and Paszczyk [42] reported a figure of 0.467 μg/kg of bw for native lake roach from the family *Cyprinidae*, representing contributions to the PTWI of 9.49% and 26%, respectively.

Based on the estimated weekly intake values obtained in the present study, the consumption of cultured carp or catfish do not represent health risks for the entire population, even with a weekly intake above 200 g (or up to 1 kg), due to the low bioaccumulation of metals in fish that are cultured in cages and fed commercial feed. Under these conditions, the fish present a contribution to PTWI < 1.5% for Cd, As, and Pb. Since Hg represents a greater health risk, and the main source of intake of this harmful metal is fish and shellfish consumption, the EWI values calculated also reflect a low risk (% PTWI < 10.0), not only for the general population, but even more so for children and pregnant woman. Consumption of native fish, in contrast, represents a moderate-to-high risk (40.89–155.36%). As a precaution, in populations with higher weekly intakes, consumption of free fish by children and pregnant women should be monitored and limited.

According to the results obtained, consuming fish raised under conditions of aquaculture in Lake Chapala does not represent a health risk for heavy metal consumption. The key to keeping fish under acceptable levels of metal concentrations was to provide metal-free supplementary feed. It is, however, advisable to monitor metal concentrations in wild fish as a means of control.

Table 6. Mean concentration (µg/kg ww), estimated weekly intake (µg/kg bw/week), and contribution to PTWI from native or cultured freshwater fish reported in previous works, compared with the present study.

Metal	Fish	Origin	Country	Mean	EWI *	% PTWI	Reference
Cd	Carp	Lake Chapala	Mexico	2.23	0.006	0.11	Present Study
	Catfish			1.23	0.004	0.06	
	Catfish	El Rejon Dam		148.00	0.395	6.58	[12]
	Cultured Carp	Lake Kasumigaura	Japan	7.40	0.021	0.35	[30]
	Wild Carp			9.00	0.026	0.43	
	Crucian carp	Honghu Lake	China	8.70	0.028	0.47	[37]
	Yellow Catfish			5.60	0.018	0.30	
	Silver Carp	Chah Nime Lake	Iran	31.20 †	0.096	1.60	[43]
	Carp	Gorgan Bay		260.00	0.743	12.38	[38]
Pb	Carp	Lake Chapala	Mexico	11.97	0.034	0.14	Present Study
	Catfish			13.03	0.037	0.15	
	Catfish	El Rejon Dam		2740.00	7.307	29.23	[12]
	Crucian carp	Honghu Lake	China	93.80	0.305	1.22	[37]
	Yellow catfish			124.20	0.403	1.61	
	Silver carp	Chah Nime Lake	Iran	47.84 †	0.47	0.59	[43]
	Carp	Gorgan Bay		430.00	1.229	4.91	[38]
As	Carp	Lake Chapala	Mexico	57.01	0.163	1.09	Present Study
	Catfish			32.39	0.093	0.62	
	Catfish	El Rejon Dam		66.00 †	0.189	1.26	[9]
	Cultured Carp	Lake Kasumigaura	Japan	178.90	0.511	3.41	[30]
	Wild carp			95.00	0.271	1.81	
	Yellow catfish	Honghu Lake	China	4.00	0.013	0.09	[37]
Hg	Carp	Lake Chapala	Mexico	3.44	0.010	0.61	Present Study
	Catfish			27.46	0.078	4.90	
	Carp			870.00	2.486	155.36	[41]
	Carp			390.00	1.114	69.64	[10]
	Carp			229.00	0.654	40.89	[11]
	Carp	San Antonio Dam		72.50	0.207	12.95	
	Catfish	El Rejon Dam		46.50	0.124	7.75	[12]
	Roach	Olsztyn Lake	Poland	140.00	0.467	29.17	[42]

* Estimated weekly intake for an adult of 70 kg or according to author, and fish consumption of 200 g. † Mean concentration adjusted to ww, 70% humidity assumed.

5. Conclusions

Observations from this study indicate that metal accumulation in fish varies markedly among different tissues and metals, but not between species, with the sole exception of Cu. Fish aquaculture in Lake Chapala is thus a viable option for producing fish with a low risk of heavy metal consumption by humans. Nonetheless, maintaining strict monitoring of metals in fish muscle is crucial because this lake is characterized by highly dynamic activity that causes frequent variations in volume and pH that affect the concentrations of metals in its waters. Finally, Hg in catfish showed the highest risk in terms of their PTWI, though even in this case, the % PTWI was below 5 %.

Author Contributions: Conceptualization, C.A.; methodology, C.A. and E.J.H.-L.; software, E.J.H.-L.; formal analysis, X.G., D.M.C.-V., and J.M.R.; resources, C.A.; data curation, writing—original draft preparation, C.A. and D.M.C.-V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Mexico's National Science and Technology Council (CONACYT), grant number 216110.

Institutional Review Board Statement: The study was conducted according to the guidelines of Mexican NOM-033-ZAG/ZOO-1994 and World Organization Aquatic Animals Health Code for slaughter of domestic animals.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors acknowledge Domingo Ausin, Jesús Arriaga, and Sergio Escobar for their support in the fish culture and sampling work.

Conflicts of Interest: The authors declare no conflict of interest.

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