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Lead and Mercury Bioaccumulation in the Fish of Floating Net Cage Fisheries

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Abstract

Saguling Reservoir has multiple roles, and functions principally as a hydroelectric power station. Other roles include supporting the fisheries and the transportation and tourism sectors. Recently, Saguling Reservoir has been widely used for freshwater fisheries. These fisheries have benefited from the development of floating net cages for fish farming in the 1980s. There are now more than 35,000 floating net cages business units or plots owned by the community in the Saguling Reservoir area. Heavy metals are among the water quality pollutants in the Saguling Reservoir. Hg and Pb are nonessential trace elements that were reported recently as serious problems in the Saguling Reservoir. These metal contents are thought to contaminate fish populations in both floating net cage and nonfloating net cage areas. For example, Hg can have a detrimental effect on the human body, involving the central nervous system and the cardiovascular system, as well as having negative effects on animal immune and reproduction systems (COWI A/S, 2002). This research was carried out in the Saguling Reservoir precisely at three different sampling points involving nonfloating net cage stations and two floating net cages stations as representative sites for heavy and moderate polluted areas, respectively. Pb and Hg heavy metal analyses were conducted in the water, fish, feed, and substrate. The heavy metal analyses were done using inductive coupled plasma-mass spectrometry (ICP-MS). The processing of heavy metal concentration data in each compartment was carried out using two-way ANOVA and Tukey post-hoc analysis. The results showed that the mean Pb concentration in sediments was 7.7925 ± 0.8575 mg/kg, which was significantly higher than the Hg concentration. On the contrary, in all fish feed brands, the Hg concentration was considerably higher than Pb. Based on the analysis, Pb accumulation in sediments might be strongly enhanced by the upstream waste industry load and leaching from inland agricultural activity, which enters the reservoir. The result showed no significant differences in Hg concentrations in fish muscle between stations, between the nonfloating net cage and floating net cages treatments. The concentration of Pb and Hg in fish muscle was lower than the permissible limit for human consumption set by the Indonesian Government and the World Health Organization.

Keywords: Lead; Mercury; Floating net cage; Reservoir; Water pollution

1. Introduction

According to Krisanti (2006), a reservoir is a water pool generally formed from rivers or swamps. Reservoirs serve special and multiple functions, but in many respects are similar to lakes; both have mass water volumes and distinct compositions and biology.

Citarum River in West Java is an example of reservoir construction in Indonesia. This river is the forerunner to the building of large reservoirs around the area. Before the existence of the Cascade Reservoir in Citarum River, Ir. H. Djuanda Reservoir was built in 1967. The Saguling Reservoir, which is located upstream of the Citarum River, was built in 1985. This reservoir is 645 m above sea level, with a total area of 5600 ha. Its depth reaches an average of 17.5 m and has a coastline length of 473 km. Cirata Reservoir was built in 1988. This reservoir is located between Saguling and Ir. H. Djuanda Reservoir. Cirata Reservoir is 221 m above sea level with an area of 6200 ha, depth of 34.9 m, and 181 m coastline (Tjahjo *et al.* 2008).

The Saguling Reservoir serves multiple roles, principally as a hydroelectric power station. The Saguling Reservoir also supports the fisheries, transportation, and tourism sectors. Since the development of floating net cages for fish farming in the 1980s, Saguling Reservoir has been widely used for freshwater fisheries. In 1986, there were just 200 floating net cages business units or plots owned by the community in the Saguling Reservoir area. By 2000, the number of floating net cages business units had increased to 48,345 scattered plots in eight districts in West Java.

Because of the multiple environmental problems threatening the sustainability of water resources, Saguling Reservoir does not have ample prospects for long-term processing. These environmental problems are principally from industrial, urban, agricultural, and fishery wastes that pollute the waters.

The Cascade Reservoir has a unique dynamic in which the quality of the waters downstream is strongly influenced by environmental management of the upstream reservoir waters. Management of the Cascade Reservoir, which supplies the Citarum River, is mainly concerned with environmental issues, both in terms of quantity and quality, due to interactions between economic activities and the preservation of water resources. The increasing number and intensity of exploitation of water resources improve the severity of aquatic ecosystem degradation involving pollution, eutrophication, the decline in biodiversity, siltation (sedimentation), and decreased productivity. These environmental problems are mainly affected by industrial, urban, agricultural, and fishery wastes. Because of this, it has been suggested that the Saguling Reservoir does not have ample prospects for long-term processing.

Heavy metal is one of the water quality pollutants in the Saguling Reservoir. Hg and Pb are nonessential trace elements that were recently reported to be a serious problem in the Saguling Reservoir. These metal contents were considered to contaminate fish populations in both floating net cage and nonfloating net cage areas. Nonessential trace elements in the edible tissues of fish, such as muscle tissue, are highly persistent and have non-biodegradable properties (Burger, 2005; Zhang, 2011; Rajeshkumar, 2018). Fish, as the top predators in the aquatic ecosystem, can accumulate heavy metals from water and sediment (Burger 2011; Zhao, 2012; Rajeshkumar, 2018) through prey that consumes organic matter directly from the sediment. There are several adverse effects of nonessential trace element toxicity in humans, as well as in animals. For example, Hg can have a detrimental effect on the human body involving the central nervous system and the cardiovascular system, while there is evidence of negative effects on the immune and reproduction systems in animals (COWI A/S, 2002).

To further investigate these problems, here we analyze the Pb and Hg levels in fish muscle tissue in the nonfloating net cage and floating net cage treatments.

2. Materials and methods

2.1 Site Description

The research was conducted downstream of Saguling Reservoir, West Java Province, Indonesia. Saguling reservoir is located downstream of the Cirata Reservoir, which is connected with the Jatiluhur Reservoir in the upper site (Figure 1).

The sampling area is located between 6°56’ and 6°58’ S. This location is used for floating-cage aquaculture. In addition, there are many activities of agricultural sites in the riparian zone that might contribute to the heavy metal load of the water body. Three stations were selected as sampling sites: station 1 located in the nonfloating cage treatment area (natural habitat) and stations 2 and 3 in floating-cage treatment areas (Figure 1). Station 1 is located near the riparian zone adjacent to the rice farming area, and has a water depth of around 6 m. Stations 2 and 3 has a water depth around 12 and 30 m respectively. This water depth differences also estimated will affect the toxicity differences between Pb and Hg.

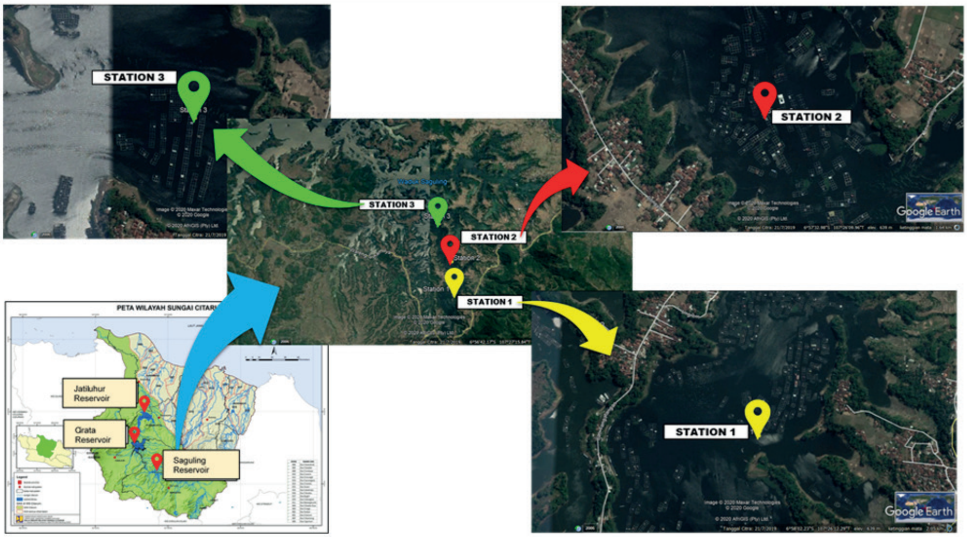


Figure 1. Sampling Location

Table 1. Sampling coordinates.

Sampling Location	**Coordinates**
Station 1	6°58'6.99"S 107°26'13.14"E
Station 2	6°57'33"S 107°26'10.00"E
Station 3	6°56'51.67"S 107°25'55.76"E

Sampling was conducted on four occasions, each separated by 11 days (Table 1). Sampling was performed during the dry season (August 10th to September 12th) in 2019. There are slight differences in the Station 2 and 3 sampling times due to natural factors, such as wind and water current, that influence the shifting of the floating net cages (Table 1).

2.2 Field Sampling

Direct measurements were conducted for dissolved oxygen (DO), pH, water velocity, and water temperature analysis. Indirect measurements were conducted for Pb and Hg analysis in the water, fish, feed, and substrate. A digital DO-meter was used for dissolved oxygen and water temperature, a digital pH-meter was used for water pH level, and a manual Styrofoam plat and stopwatch were used for water velocity. Three fish were randomly collected from each station using a fishnet and then placed in a cooler box. At each station, water sampling was conducted at depth of 2 and 4 m. The water sample was then placed into a dark plastics bottle and was replaced with a cooler box. The substrate was collected from the riparian zone around stations 1 and 2 using a wood plat and replaced to plastic and a cooler box.

2.3 Sample Preparation and Analysis

Water and substrate samples were stored in a refrigerator. Meanwhile, the fish sample was rinsed off using clean water and then kept in the freezer at -20°C after physical measurement such as wet weight (g) and length (cm). On the following day, all samples were transported to the laboratory and analyzed.

2.3.1 Sample preparation

As much as 0.5–1.0 g muscle of fish was sampled then mixed with 10 ml of concentrated HNO3. The sample was then digested using microwave digestion at 150°C before adding 0.5 ml of internal standard (yttrium, 100 mg/L). The resulting solution was diluted, homogenized, and filtered before being analyzed using the ICP-OES and ICP-MS methods. As much as 100 ml of water sample was supplemented with 1 ml HNO3 (1:1) and 5 ml HCl (1:1). Then, the solution was heated gently at 70°C until it remained at 5 ml and homogenized.

2.3.2 Sample analysis

Prepared samples of fish feed, water, substrate, and fish muscle tissue in the solution form were analyzed using inductive coupled plasma-mass spectrometry (ICP-MS) and ICP-OES (SNI 3554:2015) (Figure 2). As much as 220.353 nm and 253.650 nm of wavelength were used for Pb and Hg detection, respectively. Quality control was assured through Duplo's analysis.

2.4 Data Analysis

The differences between Hg and Pb concentrations in fish muscle between stations were compared using two-way ANOVA in SPSS (ver.23) and Tukey as post-hoc analysis. Meanwhile, the correlation between the wet weight of fish and Hg concentration levels was analyzed using a linear regression program in SPSS (ver.23).

3. Results and Discussion

3.1 The Source of Heavy Metal Pollutants in Saguling Reservoir

This section explains the sources of anthropogenic activities around the catchment area of the Saguling Reservoir, which have the potential to become a source of heavy metal pollutants in the Saguling Reservoir.

The anthropogenic activities upstream of the Saguling Reservoir watershed are various, ranging from domestic, non-domestic, industrial, agricultural, animal husbandry, and mining. The waste from all of these activities enters the Citarum River and empties into the Saguling Reservoir.

However, it is known that the main source of heavy metals in the Saguling Reservoir is derived from the activity of floating net cages. There are currently 35,482 floating net cages in the Saguling Reservoir, while the number of allowed floating net cages is around 10,000.

The main activities contributing to heavy metal pollution in the Saguling Reservoir are the provision of heavy metal-containing fish feed and fish mortality.

Agricultural activity adjacent to the Saguling Reservoir also contributed heavy metal pollution. The residual pesticides used by farmers directly enter the reservoir when carried by the flow of rain.

Industrial activities and domestic waste in the catchment area of the Saguling Reservoir make an insignificant contribution to the concentration of heavy metals in the Saguling Reservoir when compared to the activity of floating net cages and agricultural areas around the reservoir. This is because the heavy metals from upstream activities in the Saguling Reservoir have settled in the upstream river before entering the reservoir.

3.2 Physiochemical Properties of Water and Quality Standard

During the sampling period, there were two physical parameters that were measured directly in the field, water velocity and air temperature, while water level data was obtained through qualitative measurement. There are differences in water flow velocity between different locations and sampling times (Table 3), while the air temperature in all stations ranges from 27–28°C (Table 4). Those differences were affected by several factors, such as microclimate and aquatic body water typology differences.

Table 4. The condition of water flow during the sampling process.

Parameter	1st Sampling			2nd Sampling			3rd Sampling			4th Sampling		
	St.1	**St.2**	**St.3**	**St.1**	**St.2**	**St.3**	**St.1**	**St.2**	**St.3**	**St.1**	**St.2**	**St.3**
V flow (m/s)	0.11	0.13	0.33	0.08	0.09	0.14	0.09	0.05	0.05	0.07	0.14	0.08
Air Temperature	27.3	26.9	28.3	28.3	27.7	27	28	27.6	27.3	28	27.6	27.3

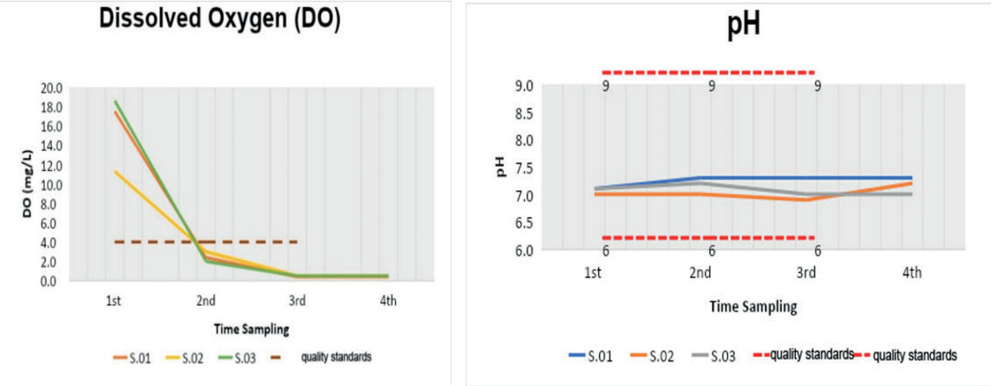


Figure 3. The outcomes of (left) dissolved oxygen (DO) and (right) water pH measurement testing on quality standard.

The water heights during sampling activity in stations 1, 2, and 3 were 4 – 6 m, 8 – 12 m, and 30–10 m, respectively. The water depth in the reservoir during the sampling process was varied; Station 1 (4 – 6 m), Station 2 (8 – 12 m), and Station 3 (10 – 30 m). The variation in water depth at each station depended on air temperature that affects the evaporation in the water surface. An advance in the rate of evaporation resulted in alterations in the water chemical-physical properties, such as changes in thermal stratification, lowered dissolved oxygen (DO), pH, light penetration (Hutabarat and Evans, 2008), and the reduction in pressure due to a drop in the depth of the water column (Nybakken, 1992).

However, chemical parameter analysis consisted of pH and dissolved oxygen (DO) measurement. These measurements were conducted directly in the field. The parameters of the analysis outcomes were then compared to PP 82/2001 quality standards on Water Quality Management and Water Pollution Control with class II labels (Figure 3). Based on PP 82 in 2001, the figure revealed dissolved oxygen levels in first sampling at stations 1, 2, and 3 exceeded the quality standard. However, based on its quality standard, the pH at each monitoring location was within safe limits or did not exceed the quality standards (Figure 3).

3.3 Pb and Hg Bioaccumulation in Sediment and Feed

Sedimentation, which formed substrate at the bottom of the water body in Saguling Reservoir, was formed by several factors, such as organic and inorganic load, including heavy metals from inland water through leaching process that is dominated by farming activity and domestic waste and fish feed from aquaculture activity (floating cage). In addition, region geology and atmospheric deposition might increase heavy metal loading into aquatic ecosystems (Nriagu and Pacyna 1988, Mukherjee 1989, Tarvainen *et al.,* 1997, Tulonen, 2006). Furthermore, fish feed from aquaculture activity was suggested to contribute to heavy metal accumulation in this area. There are four brands of fish feed that are used in floating net cages treatment in Saguling Reservoir involving Hi Provit, Sinta, Bintang, and leftover noodles from noodle factories.

Heavy metal analysis results show that the mean Pb concentration in sediment was significantly higher than Hg, which was 7.7925 ± 0.8575 mg/kg, while Hg was not detected using limit detection < 0.004 mg/kg. On the contrary, Hg concentration, which was analyzed in all fish feed brands, was considerably higher than Pb (lead), as much as 0.44 ± 0.02 mg/kg Hg concentration was detected in leftover noodle feed, which was the highest, followed by Bintang and Sinta brands (0.43 ± 0 mg/kg and 0.425 ± 0.015 mg/kg, respectively). However, the Hg concentration in the Hi Provit brand was the lowest (0.23 ± 0.01 mg/kg). In the Sinta and Hi Provit brands, the Pb contents were 0.125 ± 0.005 mg/kg and 0.015 ± 0.005 mg/kg, respectively, while Pb was not detected in Bintang or the leftover noodle feed when using the 0.0003 mg/kg limit detection (Figure 4).

Pb accumulation in the substrate might be strongly enhanced by the upstream waste industry load, especially the textile industry and leaching material from inland water, which enters the upper site of the Saguling Reservoir. The geologic conditions might determine Pb and Hg accumulation on the substrate in this area, but further investigation is needed to prove the correlation between the geologic factor and heavy metal accumulation in the sediment. The chemical characteristics of Pb, which have low solubility in water, were suggested to be the main factors driving Pb accumulates in sediment, rather than in the water column or aquatic organisms. For this reason, Pb binds strongly to the particle in the environment (COWI A/S, 2002).

3.4 Pb and Hg Bioaccumulation in Fish Muscle

Fishes sampled in this study consisted of *Cyprinus carpio, Oreochromis niloticus* (tilapia), *Hampala macrolepidota, Hemibagrus* sp., and *Pangasius* sp. Both *C. carpio* and *O. niloticus* were the dominant species in floating net cage site and also the major commodity of floating cage aquaculture. The *H. macrolepidota* and *Hemibagrus* sp. were found only in wild habitat (S.01), while *Pangasius* sp. – a secondary aquaculture commodity in Saguling floating net cage industry – were captured in S.03.

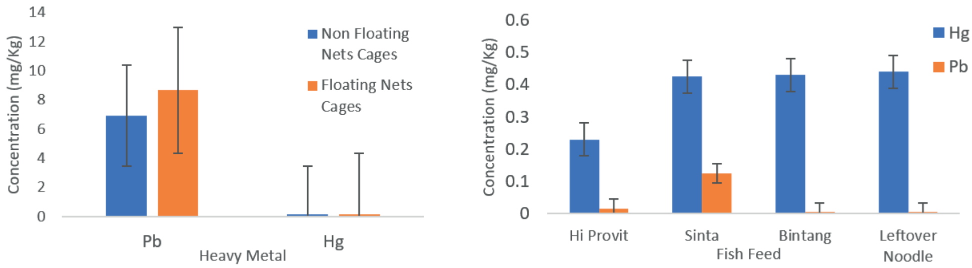
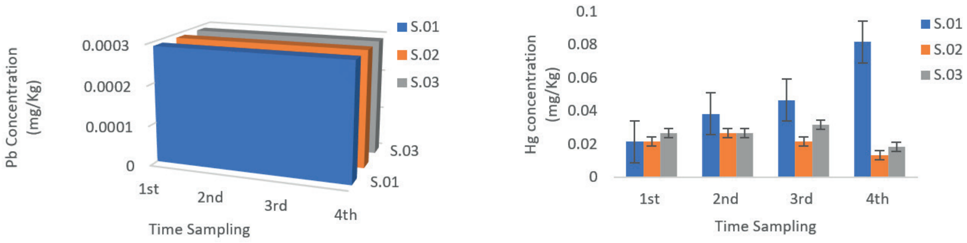


Figure 4. Pb and Hg concentrations in (left) substrate and (right) feed.



Pb

Hg

Figure 5. The Pb and Hg concentrations in the fish muscle at nonfloating net cage (S.01) and floating net cage (S.02 and S.03) sites.

We found that, in all fish samples, the Pb concentration in the fish muscle at the nonfloating net cages (S.01) and floating net cages (S.02 – S.03) were not detected using the 0.0003 mg/kg limit detection, while Hg was detected for almost all fish samples at all stations. In fish from the nonfloating cage area (S.01), Hg accumulation varied from 0.022 mg/kg to 0.082 mg/kg, which was the highest detected (Figure 5a). Lower levels of Hg were found in both floating net cage sites (S.02 and S.03), at 0.021 ± 0.006 mg/kg and 0.025 ± 0.005 mg/kg, respectively (Table 5a). These concentrations did not exceed the permissible level recommended by the national standard (BPOM) (0.5 – 1.0 mg/kg) and the World Health Organization (WHO) for human consumption.

Based on this analysis, several factors were suggested to influence the low level of Pb accumulation in fish muscle. The chemical form of Pb in the environment, which was mainly particulate bound, was suggested as the main factor for this. Based on that condition, Pb has relatively low mobility and bioavailability. In general, Pb does not bioaccumulate in most organisms but can accumulate in biota feeding primarily on particles (e.g., mussels and worms). These organisms often possess special metal-binding proteins that help to remove accumulated metals. Also, there was no increase in the concentration of the metal in the food chain (COWI A/S, 2002). For these reasons, Pb was not detected in the fish muscle, even though the Pb concentration in the sediment was high (Figure 4).

Also, fish held in floating cages, which have limited depth, are unable to prey on small fish and bottom feeders that may accumulate Pb from sediment. Bottom feeders consuming organic compounds in sediment can be predated by higher biota at higher trophic levels, potentially leading to Pb bioaccumulation in the predator. Furthermore, the continuous supply of fish feed or pellet results in the floating cage fish developing a preference for the feed pellet rather than natural prey (Figure 5a-b)

There is a gradual increase of Hg bioaccumulation among the nonfloating net caged fish and wild habitat fish during the sampling period (the dry season) (Figure 5.b). The increase in air temperature during the dry season likely leads to an increase in water turbidity. This condition will increase Hg bioaccumulation in organisms at lower trophic levels, such as bottom feeders, which are potential prey for the farmed fish. For instance, benthic invertebrates are potential prey for fish, such as carp. Its prey may contain higher concentrations of Hg during the dry season, while the Hg concentration in fish feed/pellet is unchanged. Furthermore, as a consequence of the food chain process, prey at lower trophic levels might accumulate higher Hg than found in artificial feed. As a consequence, wild fish that consume the prey have a higher Hg concentration level than in artificial treatment settings (Figure 5b).

However, environmental changes were suggested to not significantly affect the Hg bioaccumulation rate in floating net cage fish (S.02 and S.03) due to the daily provision of artificial feed. The provision of artificial feed influences the feed preferences of the fish and reduces the probability that they will hunt their natural prey. For instance, wild and floating cage carp – an omnivorous species with strong carnivorous tendencies (Crivelli, 1981; Pascal and Thierry, 1995) – have different feed preferences. Carp in the wild can swim vertically from the epilimnion to hypolimnion region and are horizontally free to seek out natural feed and prey, and tend to prey smaller organisms rather than feed death material, while fish in the floating cage have limited opportunity for movement and tend to feed the artificial pellet that provided daily. Because of that, there is no temporal variation in Hg accumulation in these fish.

Besides environmental factors, metabolism as a functional factor and membrane as a structural factor are important factors that determine bioaccumulation of heavy metal in fish. Metabolism characteristics involving enzyme systems are determined by species differences and health conditions (Lakshmanan *et al.* 2009, Rosli *et al.,* 2018). The enzyme system affects the bioaccumulation rate of heavy metals, in particular, Pb and Hg, as nonessential metals for fish. Besides that, membrane permeability differences of different species determine its ability (Lakshmanan *et al.* 2009, Rosli *et al.,* 2018) through a heavy metal selection process that then accumulates in fish tissue (Rajeshkumar and Li, 2018). In addition, differences in the accumulation of heavy metals by organisms could be a result of differences in assimilation and egestion (Egila and Daniel, 2011).

Based on our Hg analyses, the fish feed was identified as a major factor contributing to the bioaccumulation level of Hg in the muscle of wild and floating cage fish (Figure 5).

3.4.1 Statistics Analysis: The differences of Hg Bioaccumulation between stations

A two-way ANOVA analysis that compared Hg bioaccumulation between all stations resulted in a non-significant value (p = 0.081), indicating no significant difference in Hg value between stations. In addition, Tukey post-hoc analysis, which was used to determine differences of Hg concentrations among the three tested stations, returned values of 0.088, 0.166, and 0.908, respectively (Table 5b). The Tukey analysis result revealed that there is no significant difference in the Hg bioaccumulation in fish when comparing floating net cage (S.02 and S.03) and nonfloating net cage (S.01) sampling sites, or when comparing the floating net cages from the heavily polluted (S.02) and moderately polluted (S.03) areas.

Based on these results, fish feed is considered to be a major factor that directly influences the bioaccumulation of Hg in fish muscle in floating cage aquaculture and, indirectly, in wild fish.

3.4.2 Statistics Analysis: Correlation between Fish Wet Weight and Hg Bioaccumulation

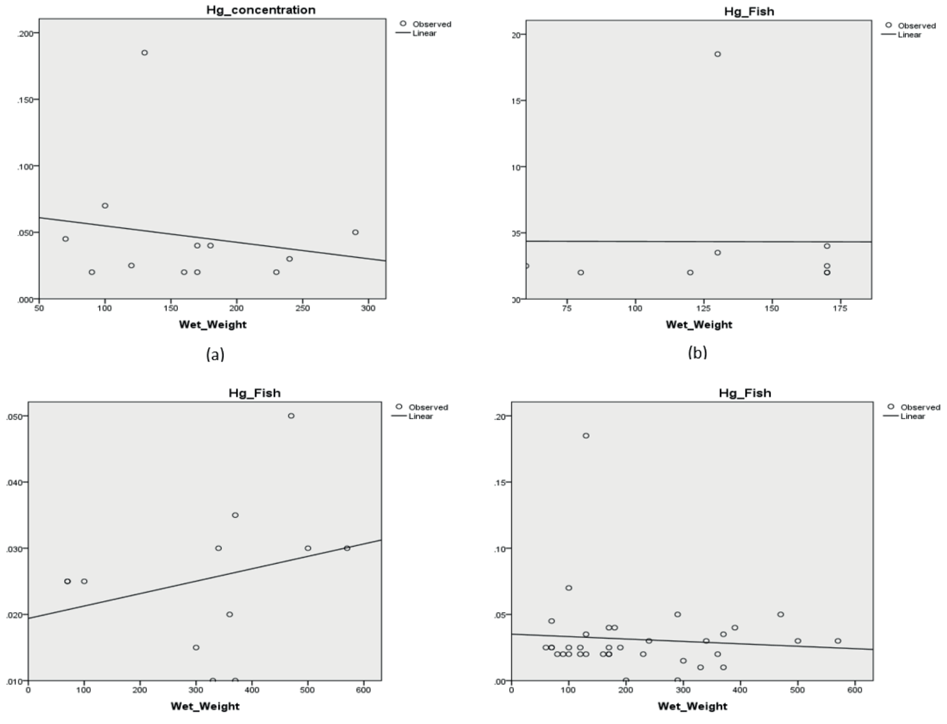
Linear regression results revealed R values and R square. R value shows correlation level between wet weight of fish while R square shows percentage of influence of fish wet weight to Hg bioaccumulation level. The correlation level values at the S.02, S.02, and S.03 sampling sites were 0.177, 0.003, and 0.277, respectively (Figure 3.5). At S.01 and S.02, there was little correlation between Hg bioaccumulation and wet weight in fish, while at S.03, a moderate correlation was detected. However, the percentages of wet

Table 5a. Hg concentrations in fish (descriptive statistics).

Station	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
S.01	4	.04708333	.025289985	.012644992	.00684132	.08732534	.021667	.081667
S.02	4	.02087500	.005452446	.002726223	.01219894	.02955106	.013500	.026667
S.03	4	.02541667	.005506730	.002753365	.01665423	.03417910	.018333	.031667
Total	12	.03112500	.018261170	.005271546	.01952241	.04272759	.013500	.081667

Table 5b. Tukey analysis of Hg concentration level in fish.

(I) station		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
S.01	S.02	.026208333	.010798430	.088	-.00394094	.05635761
	S.03	.021666667	.010798430	.166	-.00848261	.05181594
S.02	S.01	-.026208333	.010798430	.088	-.05635761	.00394094
	S.03	-.004541667	.010798430	.908	-.03469094	.02560761
S.03	S.01	-.021666667	.010798430	.166	-.05181594	.00848261
	S.02	.004541667	.010798430	.908	-.02560761	.03469094



(a) (b) (c) (d)

Figure 6. Linear regression plot of the influence of wet weight on Hg bioaccumulation at the (a) S.01, (b) S.02, (c) S.03, and (d) all sampling sites.

weight influence Hg bioaccumulation in fish muscle were 3.1% in S.01, 0% in S.02, and 0.28%. Overall, the correlation between all site sampling was 0.083, with 7% of the influence, which shows the low influence of wet weight to Hg bioaccumulation in fish tissues (Figure 6).

The variation in the level of heavy metals among different species depends upon its feeding habit, age, size, and length of the fish and their habitats (Amunsen *et al.,* 1997; Watanabe *et al.,* 2003; Rajeshkumar and Li, 2018). However, in our study, we consider feeding habits to be the main factor that influences Hg concentration levels in fish, rather than fish size and age. This is because of the continuous input of artificial feed provided directly to the fish in floating cages and, indirectly, to wild fish (that consume the leftover feed). The consistency of the artificial feed, which contains Hg, leads to similar Hg concentrations at different wet weights and ages.

4. Conclusion

Hg and Pb are nonessential trace elements that have recently been identified as a serious problem in Saguling Reservoir. These metal contents were considered to contaminate fish populations in both the floating net cage and nonfloating net cage areas. Heavy metal analysis results show that the mean Pb concentration in sediment was significantly higher than that of Hg. Pb accumulation in the sediment might be enhanced by the upstream waste industry load, especially from the textile industry and leaching material from inland water, which enters the upper site of the Saguling Reservoir. On the contrary, in all fish feed brands, Hg concentration was considerably higher than Pb. Analysis in all fish samples shows that the Pb concentration in the fish muscle at the nonfloating net cage and floating net cage were not detected using the 0.0003 mg/kg limit detection, while Hg was detected for almost all fish samples from all stations, with a low level of concentration. Based on our findings, we propose that fish feed is a major factor that directly influences the bioaccumulation level of Hg in fish muscle, both within floating cages and in wild fish.

5. Acknowledgment

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