Heavy Metal Bioaccumulation in *Wallago attu* from Peshawar: A Public Health Risk Assessment

Muhammad Ilyas Khan[*], Muhammad Abbas Hasan[†], Wali Ullah[‡], Khan Niaz Khan[§], Ali Muhammad Yousafzai[**]

Abstract

Heavy metals are defined as any metallic element that is toxic or harmful at low concentrations and have an atomic weight greater than 40 and a sufficiently high density between 3.5 and 7 g/cm3. Aquatic ecosystems and human health are under great risk due to the substantial human-caused contamination of aquaculture systems and the environment with heavy metals. The purpose of this study was to determine the levels of heavy metals present in various organs- including intestine, liver, muscles and gills of freshwater fish Mulley (Wallago attu) that were purchased at the Board Bazar, a local fish market in Peshawar, Khyber Pakhtunkhwa, Pakistan as well as the potential health concerns to humans. In the current study, three samples (n=3) of Wallago attu have been collected from the local fish market (board bazaar) in Peshawar, Khyber Pakhtunkhwa, Pakistan to determine the accumulation of the Ni, Cd, Zn and Pb in key organs (gills, Liver, Intestine and muscles) by employing Atomic Absorption Spectrometry (AAS. To assess the health implications for both fish and humans, bioaccumulation factors and health assessments were performed. The findings revealed elevated concentrations particularly in the muscles and liver, the trend was Pb > Zn > Ni > Cd. On the other hand, gills show Zn > Ni > Pb > Cd trend and intestine shows Zn > Pb> Ni > Cd trend. Zinc was found to be the most bioaccumulated metal, while Cadmium had the minimum concentration across all the studied organs of Wallago attu. Muscles were the target organ for Ni, Cd, and Pb accumulations. Zn, however, exhibited higher concentration in the gills. Results indicated that elements accumulated in the tissues of Wallago attu typically didn't surpass WHO/FAO recommendations maximum allowable level and implying no contamination and less potential risk to humans. However, it is recommended that heavy metals be continuously monitored to guarantee the safety and health of human consumption.

Keywords: Bioaccumulation, *Wallago attu*, Heavy Metals, Atomic Absorption Spectrophotometer.

[*]Department of Zoology, Islamia College Peshawar, Peshawar 25120, Pakistan, muhammadilyas785@gmail.com

[†]Corresponding author: Department of Zoology, Islamia College Peshawar, Peshawar 25120, Pakistan, mabbashassan21@gmail.com

[‡]Department of Zoology, Islamia College Peshawar, Peshawar 25120, Pakistan, walir16120@gmail.com

[§]Department of Biology Edwards College, Peshawar 25000, Pakistan, knk.edwardian@gmail.com

[**]Department of Zoology, Islamia College Peshawar, Peshawar 25120, Pakistan, amyousafzai@icp.edu.pk

Introduction

The consumption of fish has increased dramatically worldwide in recent years, particularly as more people are aware of the nutritional and health benefits of fish. Fish is a great source of protein and is also a good supply of vital minerals, vitamins, and unsaturated fatty acids (Bawuro et al., 2018). Fish and its products are vital in many aspects of a human diet, particularly for people who avoid red meat, have compromised immune systems, are undernourished, pregnant, or nursing (Zaghloul et al., 2024**).** According to the American Heart Association (AHA), including seafood in the diet two or more times a week can contribute significantly to meeting the body's omega-3 fatty acid requirements fatty acid requirements. Between 1997 and 2020, it is expected that the fish consumption in emerging countries will grow by 57%, increasing from 62.7 million tons to 98.6 million tons (Lana et al., 2014). However, fish contamination with heavy metals has raised serious concerns worldwide and is dangerous to human health. Evaluating the levels of heavy metals in fish species of economic importance is crucial for both environmental and public health monitoring because fish can absorb these metals via their diet, the surrounding water, and sediment. Consuming fish in excess or incorrectly is likely to have detrimental consequences on human health (Huang et al., 2018).

One of the main causes of environmental pollution in the world is heavy metals. As a result of industrial development, hazardous materials have been released into the environment. Once fish enter aquatic environments, heavy metals can build up in a variety of fish organs because of their stability and persistence. Fish physiology can be affected by the most prevalent heavy metals (HMs) that can contaminate the environment: chromium, arsenic, mercury, cadmium, lead, copper, and nickel. Damage to the structure and altered function of fish organs are caused by metal accumulation. Histopathological signs, oxidative stress, and modifications in transcriptional gene regulation are all experienced by fish exposed to heavy metal contamination (Kumar et al., 2023).

Heavy metal contamination in aquatic foods has received particular interest because of their capacity to collect food sources from the surrounding environment. Adsorption through tissue or skin, lipophilic tissues like gills, food particles they consume, suspended particulates they acquire from the water, and contaminated water are all common ways for fish to absorb toxic substances (Noman et al., 2022). Cu, Fe, Co, and Zn are among the elements that are crucial for fish growth and metabolism29; however, when their concentrations rise above the toxicity threshold, they may become hazardous (Xu et al., 2020). However, even at low concentrations, non-essential elements including Cd, As, Hg, and Pb have been linked to health problems in people in addition to being detrimental to aquatic life (Chowdhury et al., 2018). Research indicates that the extent of metal accumulation and its distribution across fish organs is largely species dependent. Other contributing variables includes the fish's age, sex, size, reproductive phase, locomotion patterns, feeding behavior and habitat (Bawuro et al., 2018).

It is the first study on *Wallago attu* that is sold at the Peshawar, Pakistan, board bazaar. *Wallago attu* has economic significance and is edible. The health of fish is impacted by heavy metals, which eventually lead to complications for humans. The discharge of agricultural effluents and household wastes introduces substantial amounts of heavy metals in water bodies. Consequently, a study was required to examine the levels of heavy metals in *Wallago Attu*. Thus, these were the objectives of the current study:

- To examine heavy metals in *Wallago Attu's* bodies.
- To investigate bioaccumulation of heavy metals in various organs of fish.
- To raise public awareness regarding the harmful effects of heavy metal exposure.

Methodology

Study Area

Fish from Board Bazar, a neighborhood fish market in Peshawar, which is situated at 34°N latitude and 71°E longitude, 350 meters above sea level, and has a subtropical climate, were used in the study (Ahmad et al., 2019). The fish available at the local fish market (board Bazar) in Peshawar were sourced from Kabul River and other rivers around Peshawar.

Fish Collection Procedure

Three adult *Wallago attu* samples of the same size were brought from board bazar Peshawar, Pakistan. After being brought to the lab, fish samples were cleaned using distilled water.

Fish Identification

The keys developed by Mirza and Sandhu (2006) for the identification of freshwater fishes and (Talwar & Jhingran, 1991) for the identification of Asian fishes were used to identify the collected fishes.

Fish Dissection

Prior to being dissected on a sterile glass surface, fish of comparable size were rinsed with distilled water. A stainless-steel knife was used to remove the appropriate tissues. Sections of the liver, gut, gills, muscles and skin were measured. A one-gram sample was taken from each tissue and put into a volumetric flask.

Digestion of Tissue

To check for heavy metals such as Ni, Cd, Pb, and Zn, the tissue samples were digested. After being thawed, tissue samples were washed with distilled water. Blotting factors were then used to blot the samples. Flasks were washed using distilled water and subsequently oven-dried at 60°C for 30 minutes before transferring the tissue samples. Each flask was then filled with 10 milliliters of nitric acid, wrapped in aluminum foil, and allowed to sit at room temperature for a full day. Following that, the flasks were put in a drying oven and left to digest for an hour at 160°C, producing a clear and transparent solution. Completion of digestion was signaled by the appearance of dense white vapors succeeding the release of brown fumes. Following digestion, the samples were allowed to cool before being diluted with thirty millimeters of distilled water. After passing through Whatman filter paper, the samples were placed in clean plastic bottles for storage. To detect heavy metals, all the tissue samples that were kept in bottles were taken to the University of Peshawar's major research laboratory (see Figure 1).

Figure 1: Tissues kept in bottles.

Atomic Absorption

An Atomic Absorption Spectrophotometer (Spectra AA—700 Varian, Australia) was employed to quantify the levels of Ni, Cd, Pb and Zn in the tissue specimens. Sigma-Aldrich/Merck approved reference solutions were used to calibrate the instrument. To remove contaminants, distilled water was used as a blank sample prior to analysis. A series of standard solutions were made by diluting stock solutions in order to generate calibration curves. To create calibration curves, stock solutions were diluted to create a series of standard solutions. For each metal, a five-point calibration curve was created, guaranteeing an accuracy R2 value of > 0.99. To check for instrument drift, quality control samples were examined every five sample readings. The equipment was run with optimal parameters, such as the wavelength, slit width, and lamp current for each metal.

Statistical Analysis

Three separate analyses were performed on each sample, and the findings were expressed as mg/L. Following data analysis, the mean ± S.E. was calculated using the mean and standard deviation calculation.

Results and Discussion

Heavy Metals Concentration in Wallago attu Specimen 1

The concentrations of Nickel (Ni), Cadmium (Cd), Lead (Pb) and Zinc (Zn) were analyzed in *Wallago attu's* intestine, muscles, liver, and gills (Table 1). In the *Wallago attu* specimen 1**,** the levels of Ni were higher in the intestinal tissue (0.168) than in the gills (0.109), muscles (0.069), and liver (0.115). In contrast, the gut had a greater quantity of Cd (0.063). In contrast, the gills had a higher Pb concentration (0.93) than in the muscles (0.46), liver (0.29), and intestine (0.86). Zn also displayed a higher quantity in the gut (2.034), like Ni and Cd.

Table 1: shows the actual concentrations of heavy metals in Specimen-1 of Wallago attu.

Heavy metals (mg/L)	Gills	Muscles	Liver	Intestine
Ni	0.109	0.069	0.115	0.168
Cd	0.044	0.042	0.033	0.063
Pb	0.93	0.46	0.29	0.86
Zn	0.986	0.336	0.791	2.034

Heavy Metals Concentration in Wallago attu Specimen 2

The average levels of Ni, Cd, Pb and Zn were analyzed in *Wallago attu's* intestine, muscles, liver, and gills (Table 2). Higher concentrations of Ni were found in the gills (0.191), liver (0.183), intestine (0.112), and muscles (0.099) of specimen 2 of the *Wallago attu* species. The content of Cd in the gills was greater (0.071). In a similar vein, gills had a greater quantity of Pb (1.40). Zn exhibited a greater quantity in the gills (0.797), like Cd and Pb.

Table 2: Actual concentrations of heavy metals in Specimen-2 of Wallago attu.

Heavy Metals (mg/L)	Gills	Muscles	Liver	Intestine
Ni	0.191	0.099	0.183	0.112
Cd	0.071	0.037	0.041	0.045
Pb	1.40	1.00	0.93	0.82
Zn	0.797	0.323	0.798	0.809

Heavy Metals Concentration in Wallago attu specimen 3

Specimen 3 of *Wallago attu* was measured and contrasted the real concentrations of Ni, Cd, Pb, and Zn in the intestine, muscles, liver, and gills (Table 3). The intestine had a higher concentration of Ni (0.214) than the gills (0.191), muscles (0.165), and liver (0.140), while the intestine had a higher concentration of Cd (0.063), followed by the gills (0.053), intestine (0.048), and liver (0.038). Pb was found to be more concentrated in the gills (1.54), and Zn was similarly higher in the intestine (0.955).

Table 3: Actual concentrations of heavy metals in Spiceman-3 of Wallago attu.

Heavy Metals (mg/L)	Gills	Muscles	Liver	Intestine
Ni	0.191	0.165	0.140	0.214
Cd	0.053	0.063	0.038	0.048
Pb	1.54	1.00	1.02	1.05
Zn	0.856	0.394	0.751	0.955

Average Heavy Metals Level in various organs of Wallago attu

Figure 2 and Table 4 show the comparison of average heavy metal concentrations and their standard errors across various organs of *Wallagu attu.* The intestine and gills had a higher mean Ni concentration (0.16±0.2), followed by the liver (0.14±0.01) and muscles (0.11±0.02). The gills had the highest mean concentration of Cd (0.06±0.01), followed by the liver (0.04±0.002), gut (0.05±0.01), and muscles (0.05±0.01). The gills had the highest mean Pb concentration (1.29±0.18), followed by the liver (0.74±0.22), muscles (0.82±0.18), and gut (0.91±0.07). The gut had a higher mean zinc concentration (1.2±0.38) than the gills (0.87±0.05), liver (0.78±0.01), and muscles (0.35±0.02).

Table 4: Comparison of the average concentration of heavy metals in several organs of wallago attu based on in terms of Mean ± SE.

Heavy Metals (mg/L)	Gills	Muscles	Liver	Intestine
Ni	0.16±0.02	0.16±0.02	0.11±0.02	0.14±0.01
Cd	0.05±0.01	0.06±0.01	0.05±0.01	0.04±0.002
Pb	0.91±0.07	1.29±0.18	0.82±0.18	0.74±0.22
Zn	1.2±0.38	0.87±0.05	0.35±0.02	0.78±0.01

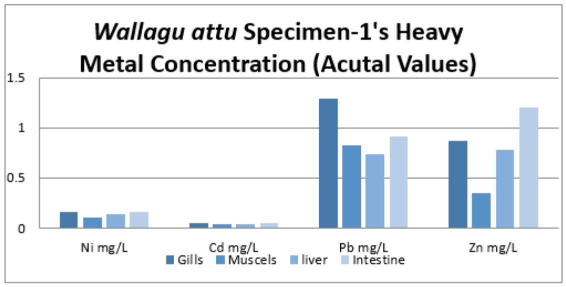

Figure 2: Average Heavy Metal Comparison.

Table 4: Comparison of the average concentration of heavy metals in several organs of wallago attu based on in terms of Mean ± SE.

Heavy Metals (mg/L)	Gills	Muscles	Liver	Intestine
Ni	0.16±0.02	0.16±0.02	0.11±0.02	0.14±0.01
Cd	0.05±0.01	0.06±0.01	0.05±0.01	0.04±0.002
Pb	0.91±0.07	1.29±0.18	0.82±0.18	0.74±0.22
Zn	1.2±0.38	0.87±0.05	0.35±0.02	0.78±0.01

Discussion

As far as we are aware**,** we carried out the initial assessment of heavy metals accumulation in fish bodies using *Wallago attu*, a type of market fish that is sold at Board Bazar in Peshawar, KP, Pakistan. *Wallago attu* was selected due to its favored nutrition and economic significance in the region. However, as the heavy metals under study include runoff from homes and farms, their selection was based on the presumed concentration levels in the study area. The intestine, muscles, liver, and gills were all tested for heavy metals (Ni, Cd, Pb, and Zn).

According to the Plaskett and Potter (1979), The Western Australian Food and Drug Regulations establish an acceptable Ni level of 5.5 mg/kg wet weight. All tissues had Pb concentrations below the threshold limit, according to the amount discovered. The Food and Drug Administration (USFDA, 2001) suggested a Cd content limit of 0.05–0.1 mg/kg for fish, while Eu established the Cd limit (2008). Based on the levels discovered, the Cd values in all fish tissues were below the threshold limit. According to the WHO (WHO, 1989) and FAO (Nauen, 1983), the highest lead levels that can be present are 0.5 and 2 mg/kg, respectively. Pb concentration in all fish tissues were below the WHO threshold limit, according to the results obtained (WHO, 1989).

The FAO/WHO states that 30 mg/kg of zinc is the highest amount that can be consumed by humans. Zinc contents in all fish tissues were below the WHO threshold limit, according to the results obtained (WHO, 1989). The bioaccumulation of heavy metals in fish is influenced by various factors such as diet, ambient temperature, water hardness, pH, salinity, age, sex and interactions between different metals (Mance, 1989; Hakanson, 1980).

Concentration of Ni in Studied Organs of Wallago attu

The 22nd most abundant element, nickel (Ni), is found in both fresh water and marine environments. Aquatic systems that get inputs from industrial and urban effluents see an increase in nickel content. It is commonly known that plants and land animals require nickel as a nutrient, whereas aquatic creatures do not. Nonetheless, there is growing evidence that Ni is most likely necessary for the fish. Fish can absorb nickel through their gills or olfactory epithelium when exposed to water or through their digestive tract when exposed to food. Nickel is carried throughout the fish's bloodstream while attached to albumins and short peptides, and it preferentially builds up in the kidneys (Pyle et al., 2011).

The mean Ni concentration in our study was highest in the gut and gills (0.16±0.2 mg/L), followed by the liver (0.14±0.01 mg/L) and muscles (0.11±0.02 mg/L). Same as our study (Al-Ghanim et al., 2016) have reported the Ni concentration among different tissues of *Wallago attu* collected from three sites: Kalabagh, Chashma and Kundian along the Indus River in Mianwali, Punjab, Pakistan. And the order of Ni concentration was gills > liver > skin > muscles. In *Wallagu attu* from river Indus, Nickel concentrations in the skin, muscles, gills and liver ranged from 0.003±0.00 to 0.708±0.00 µg/g, which were lower than those observed in the current study. Similarly, (Yousafzai et al., 2010) has been reported the Ni concentration in gills, liver, muscles, skin and intestine of *Wallago attu* from River Kabul near Nowshera, Pakistan in an order of 122.7±57.1 mg/L, 108.0±19.9 mg/L, 106.7±6.8 mg/L, 97.0±3.7 mg/L, 95.3±10.4 mg/L, respectively. Ni concentration in the gills, intestine, liver, according to research by (Siraj et al., 2014), the concentration of nickel in the gills, intestine, liver, and muscles of *Ompok bimaculatous* was in the following order: gills > intestine > liver > muscles. The mean values were 155.3±47.9 mg/L, 135.0±52.6 mg/L, and 123.7±31.5 mg/L< 100.3±66.8 mg/L.

Cd Concentrations in Studied Organs of the Wallago attu

Cd, which is extremely harmful and readily present in the air, water and other places (Maurya and Malik, 2019). Lower exposure levels than previously thought may result in cadmium exposure, which mainly manifests kidney impairment but may also cause bone problems including fractures (Jarup 2003; Maurya et al., 2016). Our results showed that the gills had the highest mean Cd concentration (0.06±0.01 mg/L), with lower levels observed in the muscles (0.05±0.01 mg/L), intestine (0.05±0.01

mg/L) and liver (0.04±0.01 mg/L) (Al-Ghanim et al., 2016) reported the maximum concentration of Cd in the skin (0.24 ± 0.00 µg/g) of *Wallago attu* collected from three sites: Kundian, Chashma, and Kalabagh along the stretch of the Indus River in Mianwali, Punjab, Pakistan. (Siraj et al., 2014) report high Cd concentration in the intestine (70.0 ± 23.5 µg/g) of *Wallago attu* from River Kabul Khyber Pakhtunkhwa, Pakistan. Similarly, a study conducted by Sthanadar, Sthanada, Yousaf, Muhammad, Zahid, (2013) reported Cd in the Gills tissue of *Wallago attu* was 0.83± 0.8317 µg/g collected from 04 different polluted sites of River Kalpani Mardan, Khyber Pakhtunkhwa, Pakistan. Similarly (Filazi et al., 2003) reported 0.15-0.50 µg/g Cd concentration in liver tissues of *Mugil auratus* collected from Sinop-Icliman in the Black Sea.

Concentration of Pb in Studied Organs of Wallago attu

Lead is a non-essential metal, as they are toxic, even in traces. Worldwide, its extensive use has resulted in health issues and environmental degradation (Rehman et al., 2015). Aquatic heavy metal concentration has a negative impact on the aquatic organism in addition to human health. Histopathological alterations in the intestinal tissue of *Wallago attu* can be caused by heavy metal concentration can be brought on by heavy metals (70.0 ± 23.5 µg/g) of *Wallago attu* from fish (Ahmed et al., 2014).

In our study the mean concentration of Pb was higher in gills(possibly due to direct waterborne exposure and metal-binding proteins in respiratory tissues) 1.29±0.18 mg/L, followed by the intestine 0.91±0.07mg/L, muscles 0.82±0.18mg/L, and liver 0.74±0.22mg/L. (Win et al., 2019) conduct a study at the Magway Township Ayeyawady River stretch, and the results show a lower mean Pb value than our data. In C. mrigala, Pb bioaccumulation varied from -0.503 mg/L to 0.717 mg/L, whereas in *W. attu*, it ranged from -0.488 mg/L to 2.450 mg/L. Only W. attu's maximum Pb value (2.450 mg/L) was marginally higher than the acceptable limit (2 mg/L) set by the WHO and FAO. Nonetheless, the average Pb level (0.865 mg/L) was below the WHO/FAO recommended limit. Mar (2019) found that the Pb levels of the fish species he studied (*Mystus avasius, Mystus leucophasis, and Wallago attu*) were 1.39 and 2.03 ppm, respectively. Our findings are consistent with this research. Cho Cho Thin’s (2017) research showed the lowest Pb concentration (-4.09 mg/l) and the highest (7.252 mg/L), both of which were greater than the results we gave.

Concentration of Zn in Studied Organs of Wallago attu

Zinc (Zn) is an essential micronutrient required in fishes (Gatlin et al., 1991) because it is closely related to many important biochemical processes, including normal growth, metabolic and immune function, gene regulation, and reproduction (Watanabe et al., 1997). Moreover, Zinc is an essential cofactor in several enzyme systems that carry out structural, catalytic, and regulatory tasks that influence nucleic acid synthesis and polysome confrontation. (Buentello et al., 2009, NRC, 2011).

In our study the mean concentration of Zn was found to be higher in gut (due to its function in enzyme metabolism and higher absorption from food) (1.2±0.38), followed by gills (0.87±0.05), liver (0.78±0.01) and muscles (0.35±0.02). (Win et al., 2019) conduct a study at Ayeyawady River segment, Magway Township report Zn values were lower than our reported data**,** with concentration ranging from 0.39 mg/L to 0.533 mg/L in *Cirrhinus mirigala* and from 0.373 m/L to 0.836 mg/L in *Wallagu attu*. Zn in the muscle tissues of studied species was lower than the permissible limit (30 mg/L) set down by WHO/FAO guidelines. (Shah et al., 2021) present the mean Zn levels in the muscles of *Schizothorax plagiostomus* sampled from different sites of the river Swat, Odigram, Charbagh and Landakai was 0.55±0.24, 0.013±0.01, and 0.08±0.06 ppm respectively which was lower than our data reported. Zn bioaccumulations in the liver of *Wallagu attu* at Kalpani River, Mardan, were 0.04 µg/g, according to (Sthanadar et al., 2013). Zn levels in fish tissues along the Yangtze River in China were 7.55 mg/kg, according to (Yi and Zhang, 2012), which was higher than our data. Because these reservoirs were contaminated by the discharge of industrial effluents, agricultural runoff, anthropogenic waste, and household waste, the concentration of heavy metals was high.

Conclusion

The current study was carried out to investigate the certain bioaccumulation of heavy metals (Ni, Cd, Pb and Zn) in the gills, muscles, intestine and liver of *Wallago attu*. The results show that the bioaccumulation of heavy metals is in order of Zn>Pb>Ni>Cd. The findings of our study also demonstrate that *Wallago attu's* intestine and gills had substantial concentrations of heavy metals. Because the intestine and the gills are the main organs via which heavy metals are absorbed in *wallago attu*. This study demonstrates that the elemental concentrations in the tissues of *Wallago attu* were typically within the acceptable limits established by WHO/FAO, suggesting that fish consumption in this region is safe for human health.

Recommendations

Heavy metals in the fish *Wallago attu* available at the local fish market of Peshawar, Pakistan did not surpass the WHO/FAO recommendation's allowable level. Therefore, when consumed, the *Wallago attu* in this study region did not provide any risks to people.

References

Ahmad, M., Khattak, M.R., Jadoon, S. A., Rab, A., Basit, A., Ullah, I. ... & Shair, M. (2019). Influence of zinc sulphate on flowering and seed production of flax (Linum usitatissimum L.): A medicinal flowering plant. *Int J Biosci*, *14*(4), 464-476.

Ahmed, M. K., Parvin, E., Islam, M. M., Akter, M. S., Khan, S., & Al-Mamun, M. H. (2014). Lead-and cadmium-induced histopathological changes in gill, kidney and liver tissue of freshwater climbing perch Anabas testudineus (Bloch, 1792). *Chemistry and Ecology*, *30*(6), 532-540.

Al-Ghanim, K.A., Mahboob, S., Seemab, S., Sultana, S., Sultana, T., Al-Misned, F., & Ahmed, Z. (2016). Monitoring of trace metals in tissues of *Wallago attu* (lanchi) from the Indus River as an indicator of environmental pollution. *Saudi Journal of Biological Sciences*, *23*(1), 72-78.

Bawuro, A. A., Voegborlo, R. B., & Adimado, A. A. (2018). Bioaccumulation of Heavy Metals in Some Tissues of Fish in Lake Geriyo, Adamawa State, Nigeria. *Journal of environmental and public health*, *2018*, 1854892.

Buentello, J. A., Goff, J. B., & Gatlin III, D. M. (2009). Dietary zinc requirement of hybrid striped bass, Morone chrysops× Morone saxatilis, and bioavailability of two chemically different zinc compounds. *Journal of the World Aquaculture Society*, *40*(5), 687-694.

Filazi, A., Sireli, M., & Kalkan, F. (2003). The influence of amitraz on biochemical parameters in mice. *Human & experimental toxicology*, *22*(2), 99-101.

Gatlin, D. M., III, O'Connell, J. P., & Scarpa, J. (1991). Dietary zinc requirement of the red drum, *Sciaenops ocellatus*. *Aquaculture, 92*(3–4), 259–265.

Hakanson, L., (1980). The quantitative impact of pH, Bio production and Hg- Contamination on the Hg-content Fish (pike). *Environ. Poll.,* 18: 285-304.

Huang, H., Li, Y., Zheng, X., Wang, Z., Wang, Z., & Cheng, X. (2022). Nutritional value and bioaccumulation of heavy metals in nine commercial fish species from Dachen Fishing Ground, East China Sea. *Scientific reports*, *12*(1), 6927.

Jarup, L. (2003). Hazards of heavy metal contamination. British Med Bull 68(1):167–82.

Kaul, B., Sandhu, R.S., Depratt, C., Reyes, F. (1999). Follow-up screening of lead-poisoned children near an auto battery recycling plant, Haina, Dominican Republic. *Environ Health Perspect.* 107(11):917–920.

Kumar, M., Singh, S., Jain, A., Yadav, S., Dubey, A., & Trivedi, S. P. (2024). A review on heavy metal-induced toxicity in fishes: Bioaccumulation, antioxidant defense system, histopathological manifestations, and transcriptional profiling of genes. *Journal of Trace Elements in Medicine and Biology*, *83*, 127377.

Mance, G. (1989). ES&T Books: Pollution Threat of Heavy Metals in Aquatic Environments. *Environmental Science & Technology*, *23*(1), 26-26.

Mar, K. M. (2019). Assessment of some heavy metals in fishes, water and effluent samples From Ayeyawady River Segment near the Fertilizer Industry. *International Journal of Biological Sciences and Technology*, *10*(1), 1-9.

Maurya, P. K., & Malik, D. S. (2019). Bioaccumulation of heavy metals in tissues of selected fish species from Ganga River, India, and risk assessment for human health. *Human and Ecological Risk Assessment: An International Journal*, *25*(4), 905-923.

Maurya, P.K and Malik, D.S. (2016). Distribution of heavy metals in water, sediments andfish tissue (Het-eropneustis fossilis) in Kali River of western U.P. India. *Int J Fisheries Aquat Stud* 4(2):208–15HUMAN AND ECOLOGICAL RISK ASSESSMENT921.

Mirza, M. R. (2006). A note on the fishes of Allai Khoar, NWFP, Pakistan. *Punjab Univ. J Zool*, *21*(1-2), 73-75.

Nauen, C. E. (1983). Compilation of legal limits for hazardous substances in fish and fishery products. *FAO Fisheries Circular (FAO). no. 764.*

Noman, M. A., Feng, W., Zhu, G., Hossain, M. B., Chen, Y., Zhang, H., & Sun, J. (2022). Bioaccumulation and potential human health risks of metals in commercially important fishes and shellfishes from Hangzhou Bay, China. *Scientific reports*, *12*(1), 4634.

NRC, N. (2011). Nutrient requirements of fish and shrimp. *Aquacult Int*, *20*, 601-602.

Plaskett, D., & Potter, I. C. (1979). Heavy metal concentrations in the muscle tissue of 12 species of teleost from Cockburn Sound, Western Australia. *Marine and Freshwater Research*, *30*(5), 607-616.

Pyle, G., & Couture, P. (2011). Nickel. In *Fish physiology* (Vol. 31, pp. 253-289). Academic Press.

Rehman, H. U., Farhan, R. A., Khattak, B., Khaliq, A., Shams, S., & Atlas, A. (2015). Estimation of lead (Pb) in the blood of road shopkeeper of KPK, Pakistan. *World Appl Sci J*, *33*, 1380-1382.

Shah, M., Kausar, S., Mian, J. A., Jabeen, H., Ullah, N., Rasool, A., ... & Ahmed, D. (2022). Bioaccumulation of heavy metals in the tissues of Schizothorax plagiostomus at River Swat. *Brazilian Journal of Biology*, *82*, e243633.

Siraj, M., Shaheen, M., Sthanadar, A. A., Khan, A., Chivers, D. P., & Yousafzai, A. M. (2014).A comparative study of bioaccumulation of heavy metals in two freshwater species, Aorichthys seenghala and Ompok bimaculatous at River Kabul, Khyber Pakhtunkhwa, Pakistan. *Journal of Biodiversity and Environmental Sciences*, *4*(3), 40-54.

Sthanadar, A. A., Sthanadar, I. A., Muhammad, A., Ali, P.A., Shah, M., Zahid, M., Begum, B. and Yousaf, M. (2013). Bioaccumulation profile of heavy metals in the liver tissues of Wallago attu (MULLEY) from Kalpani River Mardan, Khyber Pakhtunkhwa Pakistan. *International Journal of Biosciences,* vol. 3, no. 11, pp. 92-103.

Talwar, P. K., & Jhingran, A. G. (1991). *Inland fishes of India and adjacent countries* (Vol. 2). CRC press.

USFDA. third ed. US Food and Drug Administration; USA. (2001). Fish and fishery products hazards and controls guidance. *Center for Food Safety and Applied Nutrition's*.

Watanabe, T., Kiron, V., & Satoh, S. (1997). Trace minerals in fish nutrition. *Aquaculture*, *151*(1-4), 185-207.

WHO (World Health Organization). (1989). Mercury–Environmental Aspects, Environmental Health Criteria 86.

Win, D., Mar, K. M., & Khaing, T. T. (2019). Bioaccumulation of selected element concentrations in *Cirrhinus mrigala* (Hamilton-Buchanan, 1822) and *Wallago attu* (Schneider, 1801) from Ayeyawady River segment, Magway Township. *International Journal of Innovative Science and Research Technology, 4*(4), 689–694.

Xu, H., Yang, H., Ge, Q., Jiang, Z., Wu, Y., Yu, Y., Han, D., Cheng, J., (2021) Long-term study of heavy metal pollution in the northern Hangzhou Bay of China: temporal and spatial distribution, contamination evaluation, and potential ecological risk.

Environmental Science and Pollution Research 28(9):10718-10733.

Yi, Y. J and Zhang, S. H. (2012). Heavy metal (Cd, Cr, Cu, Hg, Pb, Zn) concentrations in seven fish species in relation to fish size and location along the Yangtze River. *Environmental Science and Pollution Research International,* vol. 19, no. 9, pp. 3989-3996.

Yousafzai, A. M., Chivers, D. P., Khan, A. R., Ahmad, I., & Siraj, M. (2010). Comparison of heavy metals burden in two freshwater fishes *Wallago attu* and *Labeo dyocheilus* with regard to their feeding habits in natural ecosystem. *Pakistan Journal of Zoology, 42*(5), 537–544.

Zaghloul, G. Y., Ezz El-Din, H. M., Mohamedein, L. I., & El-Moselhy, K. M. (2022). Bio-accumulation and health risk assessment of heavy metals in different edible fish species from Hurghada City, Red Sea, Egypt. *Environmental Toxicology and Pharmacology, 95,* 103969.