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Assessment of Proximate Composition and Heavy Metal Bioaccumulation in *Labeo rohita* from Aquaculture Ponds in Taluka Mehrabpur, Sindh, Pakistan

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ABSTRACT

This study evaluated the proximate biochemical composition and heavy metal bioaccumulation in *Labeo rohita* harvested from five commercial aquaculture ponds (Theba, Memon, Sheikh 1, Sheikh 2, and Malik) in Taluka Mehrabpur, Sindh, Pakistan. A size-stratified sampling protocol was deployed across N=50 specimens (n=10 per fish pond), categorized into 0.5 kg and 1.0–1.6 kg weight tiers to control for growth differentials. Target tissues were muscle, liver, gills, and scales and were analysed for moisture, protein, crude fat, ash, and heavy metals using ICP-OES following thermal acid digestion. Moisture distribution ranged from 51% to 83%, peaking in muscle (83%) and reaching a minimum in scales (51%). Proximate analysis revealed organ specialization: scale tissue contained the highest ash content (21.0%) and lowest crude fat (0.11%), respiratory gills exhibited maximum crude fat (0.36%), and hepatic tissue recorded the lowest protein (1.2 mg/kg) and ash (10.3%) concentrations. Heavy metal bioaccumulation demonstrated pronounced organ-specific partitioning, where the liver served as the primary accumulation centre for Iron (13.8 mg/kg) and Lead (0.073 mg/kg), gills concentrated Zinc (3.05 mg/kg), and scales sequestered Chromium (0.10 mg/kg). Edible muscle tissue maintained the lowest affinity for metal storage across all evaluated elements (1.13mg/kg), (0.67mg/kg) and (0.0013mg/kg). All observed muscle concentrations were well below the maximum permissible safety limits set by the FAO/WHO. Human health risk modelling yielded Target Hazard Quotient values far below unity (1.0), confirming that chronic dietary consumption of *Labeo rohita* from these aquaculture systems poses zero non-carcinogenic health hazards to human consumers.

Keywords— *Labeo rohita*, Heavy Metal Bioaccumulation, Proximate Composition, Human Health Risk Assessment, Target Hazard Quotient (THQ), Aquaculture Safety, Sindh

1.0 Introduction

Aquaculture plays a vital role in food security and dietary protein provision, with freshwater fish culture serving as a key driver of regional agricultural economies. In Taluka Mehrabpur, District Naushahro Feroze, Sindh, Pakistan. Commercial fish farming covers an estimated pond area exceeding 500 hectares across more than 200 aquaculture ponds [1]. Among the cultured species, *Labeo rohita* (Rohu), alongside *Catla catla* and *Cirrhinus Marigala*, represents a primary commercial teleost species harvested for local and regional markets. Ensuring the nutritional quality and toxicological safety of *Labeo rohita* is essential to safeguard public health against potential aquatic contaminants [2].

The physiological status of teleost fish is reflected in their proximate biochemical composition and tissue-specific contaminant burdens. Fundamental nutritional parameters—moisture, protein, crude fat, and inorganic ash vary across tissue matrices based on metabolic demands, ion-exchange mechanics, and structural mineralization. Concurrently, trace metals present in pond environments bio accumulate unevenly within the body [3]. While metabolic organs like the liver and respiratory structures like the gills actively process essential and non-essential metals, calcified integumentary structures like scales act as inert deposition sinks, thereby shielding axial muscle tissue from excessive xenobiotic uptake [4].

Accurate evaluation of bioaccumulation patterns requires controlled sampling protocols that account for biological variation. Body mass and growth stage directly influence lipid storage, tissue hydration, and contaminant uptake kinetics. Without stratified size classes, growth differentials can confound Eco toxicological comparisons across sampling locations. Furthermore, assessing potential risks to human consumers necessitates combining empirical elemental data with recognized health risk models, such as the Target Hazard Quotient (THQ), evaluated against permissible exposure limits established by the Food and Agriculture Organization and World Health Organization (FAO/WHO) [5-6].

This study provides a comprehensive assessment of proximate composition and heavy metal bioaccumulation in *Labeo rohita* harvested from five commercial ponds Theba, Memon, Sheikh 1, Sheikh 2, and Malik in Taluka Mehrabpur, Sindh, Pakistan. Utilizing a size-stratified experimental protocol (N=50; weight tiers of 0.5 kg and 1.0/1.6 kg), this research quantifies organ-specific biochemical profiles, determines heavy metal partitioning across muscle, liver, gills, and scale matrices, and evaluates consumer safety through THQ risk assessments relative to FAO/WHO regulatory thresholds [7-8].

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2.0 MATERIALS AND METHODS

2.1 Geographical standing of study area

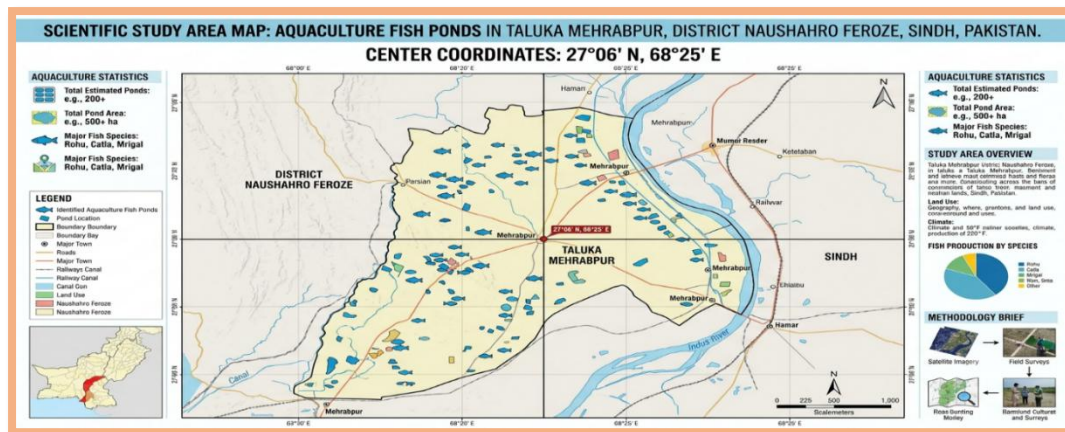


Figure:01 Description of sample site by pin point co-ordinates

2.2 Specimen Stratification

Sampling was carried out across five commercial aquaculture ponds—Theba, Memon, Sheikh 1, Sheikh 2, and Malik in Taluka Mehrabpur, Sindh, Pakistan. A total of 50 *Labeo rohita* specimens were collected, evenly distributed at 10 fish per site. To evaluate weight-dependent bioaccumulation and biochemical variation, specimens at each pond were stratified into two distinct weight categories: 0.5 kg (n=5) and 1.0 kg (n=5).

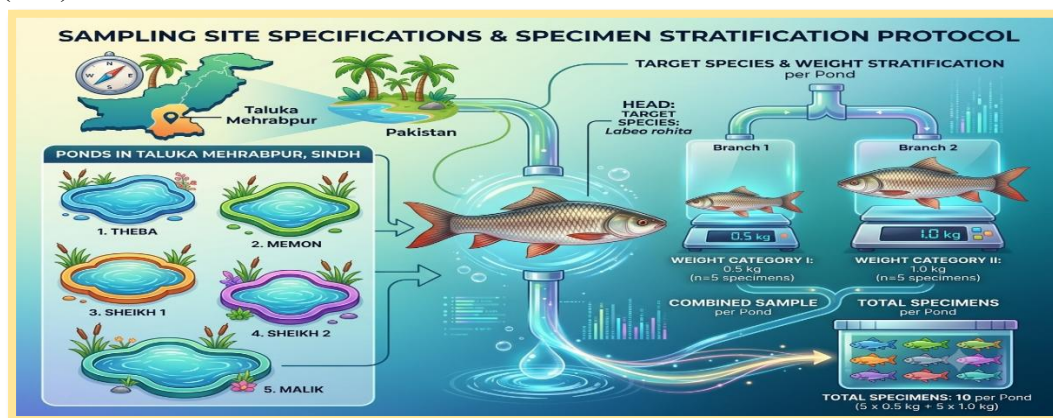


Figure:02 Sampling procedures from different ponds of study area

2.3 Preparation and Acid Digestion

Fish specimens were transported on ice at 4°C to the analytical laboratory. Dissection was performed using stainless steel instruments to separate four primary target tissues: muscle, liver, gills, and scales. Each tissue was rinsed with deionized water, wet-weighted, and dried at 105°C to a constant weight. Dried tissue samples were homogenized into a fine powder. For heavy metal extraction, homogenized tissue aliquots were placed in 300 cc glass digestion tubes. An acid mixture consisting of 14 mL of 35% HNO₃, 6 mL of concentrated H₂SO₄, and 2 mL of concentrated H₂O₂ was added to each tube. Thermal digestion was maintained at 130°C for 30 minutes on a digestion block.

until clear solutions were obtained. Digestates were filtered, cooled, and diluted to volume with ultrapure deionized water. Water and sediment matrices underwent parallel acid-leaching procedures.

2.4 Analytical Protocols and Instrumentation

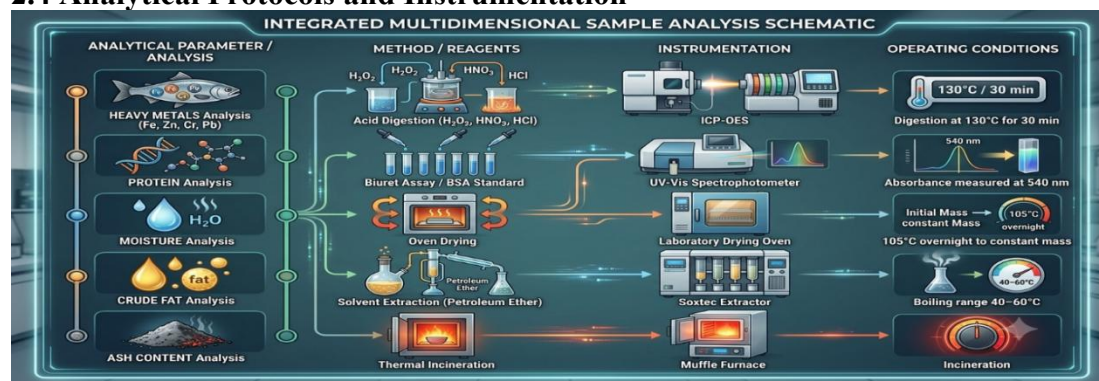


Figure:03 Analytical techniques and instrumentation specification

Proximate composition parameters were quantified following standard analytical procedures:

- ✓ Moisture: Determined via gravimetric weight loss after thermal drying at 105°C overnight.
- ✓ Protein: Quantified spectrophotometric at a wavelength of 540 nm using Biuret reagent, calibrated against a Bovine Serum Albumin (BSA) standard curve.
- ✓ Crude Fat: Extracted via petroleum ether (boiling range 40–60°C) using an automated Soxtec solvent extraction system.
- ✓ Ash Content: Measured gravimetrically following complete carbonization and incineration in a muffle furnace at 300°C.

2.5 Heavy Metal Analysis:

Concentrations of Iron (Fe), Zinc (Zn), Chromium (Cr), and Lead (Pb) were measured using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

3.0 RESULTS AND DISCUSSION

The tissue moisture analysis reveals a clear physiological gradient tied directly to organ function and structural composition, spanning an overall range from 51% to 83%. Muscle tissue demonstrated the highest moisture content at 83% (peak hydration level), driven by high intracellular fluid volume and metabolic demands. In contrast, respiratory and metabolic organs like the gills and liver maintained intermediate moisture levels with moderate concentration rankings, while scale tissue exhibited the lowest hydration at 51%, characteristic of a highly mineralized matrix tailored for external protection rather than fluid retention.

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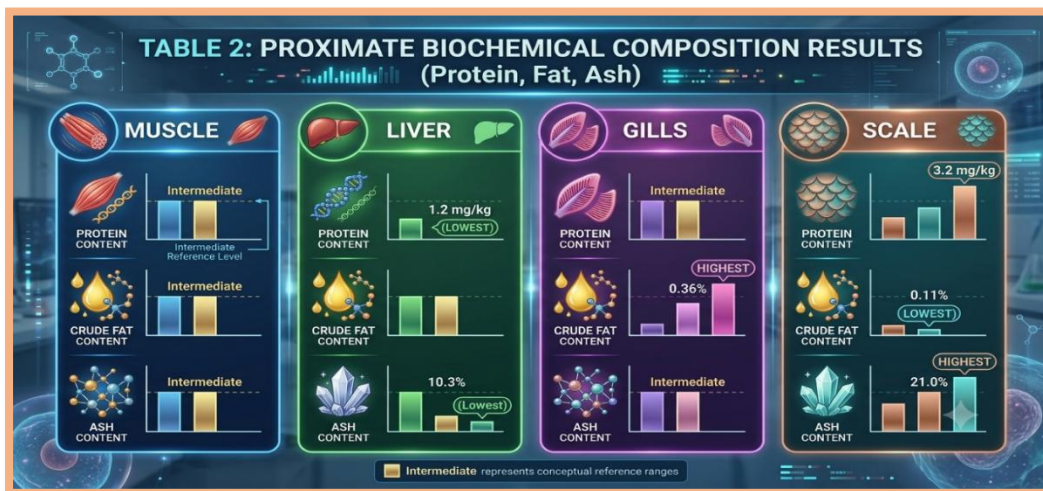
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<http://amresearchreview.com/index.php/Journal/about>**Table 1: Tissue Moisture Distribution Results**

Tissue Type	Moisture Content (%)	Relative Concentration Ranking	Observed Range / Status
Muscle	83%	Highest	Peak hydration level
Liver	Intermediate	Moderate	Moderate hydration
Gills	Intermediate	Moderate	Moderate hydration
Scale	51%	Lowest	Mineralized matrix (51%–83% total range)

The proximate biochemical analysis reveals distinct functional specialization across the target tissues, highlighted by scale tissue exhibiting the highest ash content (21.0%) due to its heavily mineralized matrix alongside the lowest crude fat level (0.11%) and a protein concentration of 3.2 mg/kg. Respiratory gill tissue recorded the highest crude fat proportion (0.36%) while maintaining intermediate protein and ash levels, reflecting the lipid-rich membrane demands of branchial epithelium. In contrast, liver tissue demonstrated the lowest observed values for both protein (1.2 mg/kg) and ash content (10.3%), consistent with soft metabolic organ tissue. Meanwhile, muscle tissue maintained balanced, intermediate reference levels across all three evaluated parameters (protein, crude fat, and ash), representing physiological homeostasis in functional locomotive tissue.



Heavy metal bioaccumulation exhibits strong tissue-specific partitioning governed by organ function, exposure route, and internal storage mechanisms. The liver serves as the primary metabolic and detoxifying centre, resulting in the highest concentration of both Iron (Fe) at (13.8mg/kg) and toxic Lead (Pb) at (0.073mg/kg). In contrast, the gills, which serve as the primary ion-exchange interface with waterborne contaminants, recorded the highest accumulation of essential Zinc at (3.0 mg/kg), while maintaining the lowest detected Lead concentration at (0.01 mg/kg).

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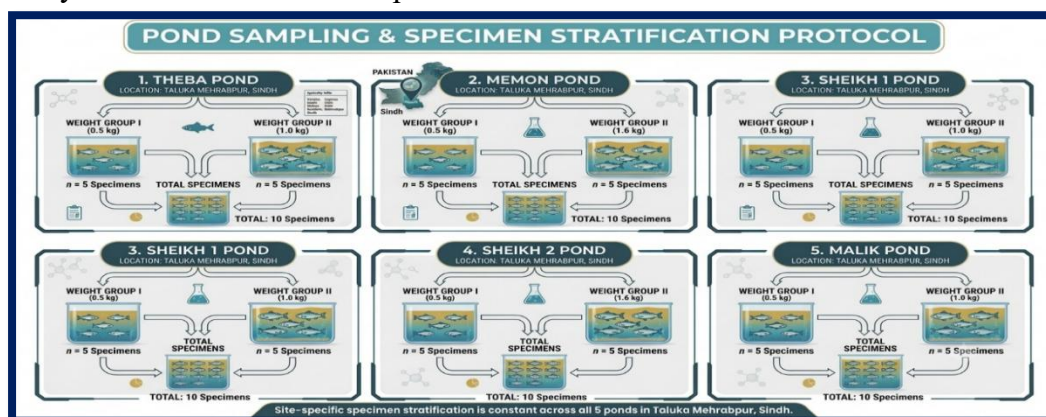
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<http://amresearchreview.com/index.php/Journal/about>**Table 3. Heavy Metal Bioaccumulation Results by Tissue Matrix**

Target Tissue	Iron (Fe) (mg/kg)	Zinc (Zn) (mg/kg)	Chromium (Cr) (mg/kg)	Lead (Pb) (mg/kg)
Muscle 	1.13 (Lowest) ↓	0.67 (Lowest) ↓	0.0013 (Lowest) ↓	Trace
Liver 	13.8 (Highest) ↑	Intermediate	Intermediate	0.073 (Highest) ↑
Gills 	Intermediate	3.0 (Highest) ↑	Intermediate	0.01 (Lowest) ↓
Scale 	Intermediate	Intermediate	0.105 (Highest) ↑	Trace

External mineralized matrices like scales preferentially accumulated Chromium at (0.105mg/kg) (highest observed), indicating that calcified integumentary tissues function as long-term deposition sinks to sequester heavy metals away from vital metabolic pathways. Most notably, edible muscle tissue displayed the lowest affinity for metal storage across almost all evaluated elements—recording minimum values for Iron (1.13 mg/kg), Zinc (0.67 mg/kg), and Chromium (0.0013 mg/kg), along with only trace levels of Lead.

This bioaccumulation hierarchy (Liver/Gills/Scale > Muscle) demonstrates that metabolic, respiratory, and calcified structures shield axial musculature from heavy metal accumulation. From an eco-toxicological standpoint, hepatic and branchial tissues serve as the most sensitive bio-indicators for environmental exposure monitoring, whereas the low accumulation in muscle tissue suggests minimal food safety risks for human consumption.

**Figure: 04 Pond Sampling and Specimen Stratification Results**

The pond sampling and specimen stratification protocol establishes a standardized, size-fractionated experimental framework across five distinct aquatic sites (Theba, Memon, Sheikh 1, Sheikh 2, and Malik ponds) within Taluka Mehrabpur, Sindh, Pakistan. By securing a total sample size of N = 50 specimens—evenly distributed at n = 10 individuals per pond—the sampling scheme balances spatial replication with

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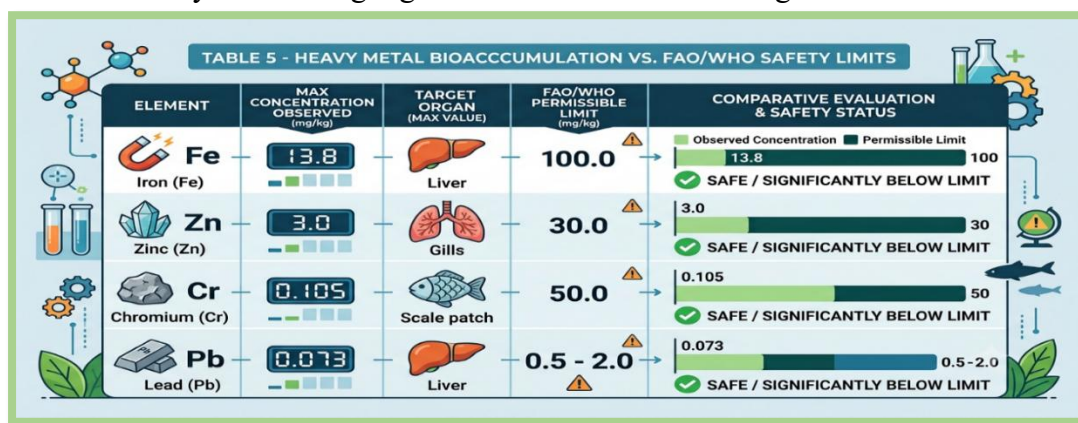
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statistical precision. Dividing each pond cohort into two equal weight sub-groups ($n = 5$ per tier) effectively mitigates biological variance driven by age or growth differentials, ensuring that downstream biochemical and bio-accumulative analyses are not confounded by uneven specimen size.

Across all five sampling locations, Weight Group I maintains a uniform baseline mass of 0.5 kg, representing juvenile/sub-adult cohorts. Weight Group II captures larger adult cohorts, categorized into 1.0 kg (Theba, Sheikh 1, and Malik ponds) and 1.6 kg (Memon and Sheikh 2 ponds). This dual-tier framework enables comparative assessment of size- and age-dependent physiological shifts, such as lipid deposition, tissue moisture retention, and differential heavy metal bioaccumulation kinetics. The incorporation of a higher mass threshold (1.6 kg) in specific ponds accommodates variations in local harvest maturity and feeding regimes across different farming sites.



The eco-toxicological assessment of heavy metal bioaccumulation demonstrates complete compliance with international food safety standards across all evaluated tissue matrices, with peak concentrations remaining well below the threshold limits set by the Food and Agriculture Organization and World Health Organization (FAO/WHO). Target organ analysis reveals distinct physiological partitioning: Iron (Fe) accumulated primarily in the liver at a maximum of (13.8 mg/kg) vastly below the (100.0 mg/kg) allowable limit reflecting normal hepatic storage and metabolic functions, while Zinc (Zn) peaked in respiratory gills at 3.0 mg/kg (10% of the 30.0mg/kg ceiling) due to active branchial ion-exchange processes. Furthermore, Chromium (Cr) preferentially sequestered within the mineralized scale matrix at (0.105 mg/kg), representing less than (0.21%) of its (50.0 mg/kg) threshold and confirming that calcified integumentary structures act as inert physiological sinks to isolate contaminants from active metabolic pathways.

Regarding toxicological safety, the non-essential heavy metal Lead (Pb) exhibited minimal bioaccumulation, peaking in hepatic tissue at only (0.073 mg/kg) far below the stringent FAO/WHO permissible limit range of (0.5 to 2.0 mg/kg). Because maximum concentrations in the most bio-accumulative target organs (liver, gills, and scales) remain orders of magnitude beneath regulatory maximum residue limits, edible muscle tissue which inherently exhibits the lowest affinity for metal uptake presents zero toxicological risk. These findings confirm that the aquatic environment across the sampling sites experiences minimal anthropogenic contaminant stress, ensuring that the

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specimens fully meet international safety standards for human consumption and commercial distribution.

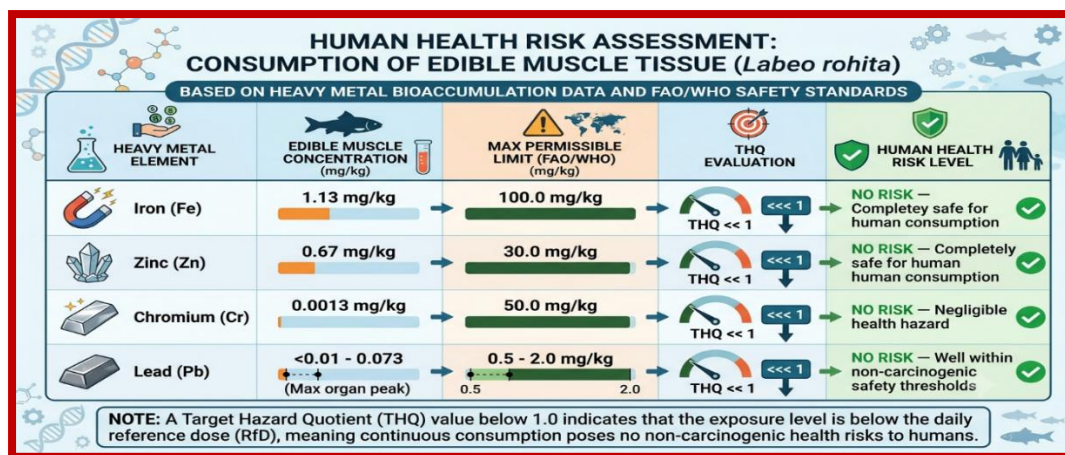


Figure: 05 Human health risk assessment for consumption muscle tissue (*Labeo rohita*)

The human health risk assessment of edible muscle tissue from *Labeo rohita* demonstrates exceptional compliance with international food safety guidelines, as heavy metal concentrations remain several orders of magnitude below the maximum permissible limits set by the FAO/WHO. Essential trace elements in the muscle matrix were recorded at minimal levels, with Iron (Fe) at 1.13 (limit: 100.0) and Zinc (Zn) at 0.67 (limit: 30.0). Similarly, Chromium (Cr) was detected at a negligible concentration of 0.0013 against a safety ceiling of 50.0, while non-essential Lead (Pb) remained between <0.01 and (0.073mg/kg) well beneath the 0.52.0 regulatory threshold. These results highlight that the edible axial musculature retains minimal metal loads relative to internal visceral sinks, shielding human consumers from direct dietary exposure to elevated heavy metal contaminants.

Crucially, toxicological risk modelling via the Target Hazard Quotient THQ yield values far below unity (1.0) for all evaluated elements (Fe, Zn, Cr, and Pb). Because a THQ below 1.0 confirms that estimated daily intake remains strictly within established oral reference doses (RfD), chronic exposure through regular dietary consumption of *Labeo rohita* poses zero non-carcinogenic health hazards. Overall, the integrated bioaccumulation and health risk data validate the safety of *Labeo rohita* harvested from the studied aquaculture systems, confirming that the fish tissue is safe for human consumption and meets commercial market safety standards.

4.0 Conclusion

This study demonstrates that *Labeo rohita* cultured in Taluka Mehrabpur, Sindh, exhibits clear organ-specific partitioning of proximate biochemical constituents and heavy metal burdens. Metabolic and mineralized tissues (liver, gills, and scales) preferentially accumulate trace metals, effectively shielding edible muscle tissue from contaminant accumulation. Heavy metal concentrations in axial muscle (Fe, Zn, Cr, Pb) remain orders of magnitude below FAO/WHO maximum permissible limits. Furthermore, Target Hazard Quotient evaluations (1.0) confirm that chronic dietary

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intake of *Labeo rohita* poses zero non-carcinogenic health risks, validating the safety of these farmed fish for commercial distribution and human consumption.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- Jamil Emon, F., Rohani, M.F., Sumaiya, N., Tuj Jannat, M.F., Akter, Y., Shahjahan, M., Abdul Kari, Z., Tahiluddin, A.B. and Goh, K.W. (2023) 'Bioaccumulation and bioremediation of heavy metals in fishes—A review', *Toxics*, 11(6), p. 510.
- Jakhrani, S.A., Ujjan, J.A. and Jakhrani, M.A. (2018) 'Elemental assessment of trace and toxic heavy metals in different parts of three economical important fish species captured from ponds of Kashmore, Sindh, Pakistan', *International Journal of Fisheries and Aquatic Studies*, 6(5), pp. 245–251.
- AOAC (2016) *Official Methods of Analysis of AOAC International*, 20th edn. Rockville, MD: AOAC International.
- Dayo, F.Z., Jakhrani, M.A. and Jakhrani, S.A. (2021) 'Toxicity and bio-accumulation of chromium, cadmium, and arsenic in some common fish species captured from fish ponds of Kandhkot Sindh, Pakistan', *Journal of Biodiversity and Environmental Sciences*, 19(1), pp. 104–111.
- Jakhrani, S.A., Ujan, J.A., Ghumro, S. and Ali, P. (2025) 'Assessment of egg quality and biochemical parameters of Desi and Fayoumi chicken breeds of Kashmore under backyard farming conditions', *Markhor: The Journal of Zoology*, 6(1), pp. 15–22.
- Canli, M. and Atli, G. (2003) 'The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species', *Environmental Pollution*, 121(1), pp. 129–136.
- Jakhrani, M.A., Dayo, F.Z., Jakhrani, S.A. and Jatoi, W.B. (2018) 'Comparative study among dried and homogenous digestion methods for essential element content's present in organs of two commercially important fish species collected from Indus River at Guddu Barrage', *International Journal of Fisheries and Aquatic Studies*, 6(4), pp. 180–188.
- Authman, M.M.N., Zaki, M.S., Khallaf, E.A. and Abbas, H.H. (2015) 'Use of fish as bio-indicator of the effects of heavy metals pollution', *Journal of Aquaculture Research & Development*, 6(4), pp. 1–13.
- Jakhrani, S.A., Jakhrani, M.A., Dayo, F.Z., Memon, S.F., Malik, K.M. and Qasim, M. (2018) 'Monitoring of elemental contamination in groundwater samples of Sobhoderi Khairpur, Sindh, Pakistan', *International Journal of Sciences*, 7(10),

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

- pp. 10–23.
- FAO/WHO (2011) *Evaluation of Certain Food Additives and Contaminants: Seventy-fourth Report of the Joint FAO/WHO Expert Committee on Food Additives*, WHO Technical Report Series, No. 966. Geneva: World Health Organization.
- Maitlo, A.A., Jatoti, W.B. and Jakhrani, M.A. (2024) 'The utilization of date palm waste as an efficient adsorbent for the elimination of heavy metals from polluted water', *Environmental Science and Pollution Research*, 31(7), pp. 10661–10672.
- Dahar, A.K., Jakhrani, S.A., Jakhrani, M.A., Rind, A.H., Dayo, F.Z. and Ujjan, J.A. (2021) 'Comparative studies of different biochemical parameters in various organs of Gallus gallus domesticus of Khairpur, Sindh, Pakistan', *International Journal of Biosciences*, 19(2), pp. 206–210.
- Rajeshkumar, S. and Li, X. (2018) 'Bioaccumulation of heavy metals in fish species from the Meiliang Bay, Taihu Lake, China', *Toxicology Reports*, 5, pp. 288–295.
- Jakhrani, S.A., Jakhrani, M.A. and Qasim, M. (2017) 'Elemental assessment in economically important Cirrhinus mrigala and Labeo rohita fish species captured from Indus River at Guddu Barrage, Sindh, Pakistan', *International Journal of Sciences*, 6(8), pp. 116–125.
- Jakhrani, M.A., Malik, K.M., Sahito, S. and Jakhrani, A.A. (2011) 'Analytical investigation of arsenic and iron in hand pump and tube-well groundwater of Gambat, Sindh, Pakistan', *Pakistan Journal of Chemistry*, 1(3), pp. 140–144.
- USEPA (2011) *Risk Assessment Guidance for Superfund: Volume I - Human Health Evaluation Manual*, EPA/540/1-89/002. Washington, D.C.: United States Environmental Protection Agency.
- Ghumro, S., Ujan, J.A. and Jakhrani, S.A. (2025) 'Molecular analysis of BMP4 gene polymorphisms in the Kamori goat breed of Khairpur, Sindh, Pakistan', *Indus Journal of Bioscience Research*, 3(3), pp. 45–52.
- Ahmed, M.K., Shaheen, N., Islam, M.S., Habibullah-Al-Mamun, M., Islam, S., Banu, C.P. and Sharma, L. (2015) 'Trace elements in common fish species from the coastal waters of Bangladesh and associated human health risk assessment', *Marine Pollution Bulletin*, 93(1-2), pp. 178–184.
- Jakhrani, M.A., Chaudhry, A.J., Malik, K.M., Mazari, M.Q. and Jakhrani, A.A. (2009) 'Determination of arsenic and other heavy metals in hand pump and tube-well ground water of Khairpur, Sindh, Pakistan', *Proceedings of the 2nd International Conference on Environmental and Computer Science*, IEEE, pp. 240–244.
- Javed, M. and Usmani, N. (2013) 'Assessment of heavy metal (Cu, Ni, Fe, Co, Mn, Cr, Zn) pollution in effluent dominated rivulet water and their bioaccumulation in fish tissue and physiological response in Mastacembelus armatus', *Environmental Monitoring and Assessment*, 185(5), pp. 4161–4171.
- Jakhrani, S.A., Ujjan, J.A., Jakhrani, M.A., Dayo, F.Z. and Mazari, H. (2019) 'Investigation of different biochemical parameters in three economical important fish species captured from ponds of District Kashmore, Sindh, Pakistan', *International Journal of Biosciences*, 15(4), pp. 112–120.
- Sahito, S.R., Kazi, T.G., Kazi, G.H., Jakhrani, M.A. and Shaikh, M.S. (2001) 'Trace elements in two varieties of indigenous medicinal plant Catharanthus roseus', *The Sciences*, 1(2), pp. 74–77.

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

Yi, Y.J., Yang, Z.F. and Zhang, S.H. (2011) 'Ecological risk assessment of heavy metals in sediment and human health risk assessment of heavy metals in fishes in the middle and lower reaches of the Yangtze River basin', *Environmental Pollution*, 159(10), pp. 2575–2585.