

ANALYZING CADMIUM AND NICKEL LEVELS IN *LABEO ROHITA*, *CATLA*, *CATLA*, AND *CIRRIHINUS MRIGALA* FROM LOCAL FISH MARKETS IN LAHORE, PAKISTAN

KHADIJA ILYAS¹, SANA SHAHBAZ^{1*}, MUHAMMAD MATEEN TAHIR², MUHAMMAD NAVEED³, AMMARA RIAZ⁴, MUHAMMAD NASEEM ULLAH⁵, MUHAMMAD WAHEED⁶

¹Department of Zoology, Lahore Garrison University, Lahore, Pakistan

²Key Laboratory of Molecular Epigenetics, Ministry of Education and Institute of Cytology and Genetics, Northeast Normal University, Changchun 130024, China

³Key Laboratory of Geographical Processes and Ecological Security in Changbai Mountains, Ministry of Education, School of Geographical Sciences, Northeast Normal University, Changchun 130024, China

⁴Department of Life Sciences, Faculty of Natural and Applied Sciences, Khwaja Fareed University of Engineering and Information Technology

⁵Department of Zoology, Virtual University of Pakistan, Sahiwal campus

⁶Punjab school Education Department, Government Higher Secondary School Nangal Sahdan Muridke 39000, Sheikhpura, Pakistan

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KI contributed to experimental design, SS supervised the research, MMT writing and editing the manuscript. MN performed Statistical Analysis, AR contributed to data acquisition, MNU and MW contributed to manuscript preparation.

Key words:

Cadmium, Nickel, *Labeo rohita*, *Catla catla*, *Cirrihinus mrigala*

ABSTRACT

Thirty-six (36) fish samples were collected from the different local fish markets of Lahore city to check the bioaccumulation of heavy metals. The mean Cd concentration ranged from (32.33 – 39.69ug/kg), (23.26 – 35.45ug/kg), (35.37 – 45.06ug/kg) in gills (28.99– 38.17ug/kg), (21.74– 30.07ug/kg), (33.92 – 43.61ug/kg) in muscles (26.12– 35.56ug/kg), (18.87– 31.32ug/kg), (31.05 – 41.01ug/kg) in liver of *Labeo rohita*, *Catla catla* and *Cirrihinus mrigala* respectively. The mean Ni concentration ranged from (44.93 – 52.86ug/kg), (45.65 – 52.86ug/kg), (52.30 – 61.14ug/kg) in gills (43.48– 51.41ug/kg), (44.20– 54.62ug/kg), (50.86 – 59.69ug/kg) in muscles (40.48 – 48.81ug/kg), (41.56 – 48.81ug/kg), (48.21 – 57.08ug/kg) in liver of *Labeo rohita*, *Catla catla* and *Cirrihinus mrigala* respectively. The comparison between sampling markets revealed no significant difference among them and the results between fish species shows highest concentration of Ni (55.26ppb) in *Cirrhina mrigala*. The accumulation pattern of all the heavy metals in different organs of all the studied fish species was significantly lower ($p < 0.05$) as compared to admissible concentration reported by WHO. Therefore, the fish of these areas do not pose serious threat to human health upon consumption.

1. INTRODUCTION

The major problem for the permanent stay of heavy metals in aquatic flora and fauna is due to its ability of bio concentration and biomagnifications which is responsible for the accumulation of heavy metals in living bodies at prominent level (Agah, 2021). Heavy metals including chromium, manganese, iron, cobalt, nickel, zinc, copper, and selenium etc. are very important to living bodies whereas lead, cadmium, mercury, and arsenic are dangerous for human health (Ozturk et al., 2009).

Even essential trace metals are dangerous for health if their concentration is slightly greater than their recommended values (Sapozhnikova et al., 2005). Fish is considered the major source of protein. All over the world human beings get twenty five percent (25%) of proteins from seafood (Benson et al., 2007). According to rough estimate in 2004, humans consumed almost 75%, that is 105.6 million tons of world level fish production (Rakib et al., 2021). It has been predicted that fish consumption in developing countries will increase by 57 percent, from 62.7 million tons in 1997 to 98.6 million in 2020 (Carraso et al., 2008).

*Corresponding Author: dr.sanashahbaz@gmail.com

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Omega-3 fatty acids, eicosapentenoic acid (EPA) and docosahexenoic acid (DHA) are richly found in Fish, shellfish and prawns. This reduces heart diseases, cholesterol level, and preterm delivery in humans (Agah, 2021). The wastewater effluents discharged from factories is responsible for Minamata disease which is because of eating mercury contaminated seafood (Carrasco et al., 2008). Ita-Ita disease in humans of Japan was also considered because of consuming cadmium contaminated seafood which was the result of digging in mines in the proximate area (Benson et al., 2007).

Acute heavy metals toxications can destroy brain and heart functioning and GIT track including liver and endocrine gland in human beings. Chronic exposure of Pb (II) may lead to the death (Benson et al., 2007). As the ions of trace metal in human beings is mainly due to consumption of heavy metal polluted diet. Thus, the heavy metal analyses of eatables consumed by humans are performed on regular basis. Because fish is present at the apex level of aquatic food chain, so large number of heavy metals are biomagnified making it dangerous to eat (Qadir & Malik, 2011).

2. MATERIALS AND METHODS

The research was conducted at Lahore Garrison University Lahore and some part of the Experimental work was performed at the Lab of the LCWU (Lahore College for women university, Lahore).

Study Area

Thirty-six (36) fish samples were collected from the different local fish markets of Lahore city to check the bioaccumulation of heavy metals. Due to the intensive agricultural conditions, unchecked industrial waste, global warming, heavy traffic, and urbanization, these fish markets were the most polluted areas. Due to the high contamination of industrial waste, heavy metal is exposed to be present in large quantity, so these sites are selected for study (Iqbal et al., 2013).

Collection of fish

During March 2021, a total of thirty-six (36) samples of *Labeo rohita*, *Catla catla* and *Cirrhina mrigala* were collected from different local fish markets (Bhatti Chowk, Shahdara and Do Moria Pull) of Lahore city. These fish samples were transferred to the lab, clean with water and then stored at -20 °C. During sample collection, the weight of each fish sample was recorded by using weight machine.

Dissection of sample

Fish were dissected under laminar air flow by using sterilized apparatus and different tissues like muscles, gills and liver were separated and washed with de-ionized

water. Dissection of fish sample was done and separated the desired parts as liver, Gills and Muscle. The muscle sample was obtained by careful removal of operculum, epidermal and subcutaneous tissues. The liver was isolated by separating it through connective tissues surrounding the liver. Washing was done by distilled water. After washing samples were labelled by specific tag. Dissected samples were stored at 20° C for further analysis (Kvach et al., 2016).

Instrumentation

Inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin Elmer Optima 7000DV) was used for analysis of heavy metal concentration in fish and water samples. Using ICP-OES, samples were subjected as acid solutions to be nebulized and formed a fine aerosol that was transported into the plasma. Then, it undergone desiccation, vaporization to molecular gases, and dissociation into atoms that could be ionized. Dilutions were made from stock solution and standard solution for the detection of metals was prepared (Sharma, 2020).

Glassware and reagents

De-ionized water was used during the whole working in lab for the washing purpose of all the apparatus. Samples bottles were rinsed with HNO₃ before the collection of samples. To avoid any contamination all glassware was washed with HNO₃ several times (Voegborlo & Adimado, 2010).

Preparation of fish tissues for Wet Digestion

- 1- Liver, kidney, muscles, and gills of fish were thawed and weighed in measuring balance. Each sample of 2g was taken and chopped. It was chopped with a sterilized steel knife and mixed properly to achieve homogeneity.
- 2- In china crucible 10ml of 1:1 HNO₃ was taken and mixed with fish solid sample and allow to heat on hot plate at 90 to 95 °C and for 10 to 15 minutes it was refluxed.
- 3- On the cooling of sample 3ml of 30% hydrogen peroxide (H₂O₂) and 2ml of deionized water was added. Samples were placed again onto the hot plate and allowed to heat until the effervescence stops.
- 4- Again 1ml of 30% H₂O₂ was added and warmed until the effervescence stop and cooled at room temperature.
- 5- By using the syringe filter, Nalgene 0.25 µm the digested samples of fish tissues were filtered. Filtrate was added to a new glass vial and stored at room temperature until analytical procedures could be done (Idera et al., 2015).

Analysis of Samples for Determination of Metal ions

The above-mentioned samples (filtrates) were sent to lab of Microbiology and genetics LCWU, Lahore, for heavy metal determination by Cadmium (Cd), Nickel (Ni), by using “inductively coupled plasma optical emission

spectrometry (ICP-OES, Perkin Elmer Optima 7000DV)” to estimate the heavy metal contents in trace quantities (Mensoor & Said, 2018).

Statistical Analysis

By using statistical software (SPSS) for social sciences, statistical analysis of the data was performed. One way ANOVA and Microsoft Excel 2019 were performed for the determination of significant difference between the values.

3. RESULTS AND DISCUSSION

Concentration of different heavy metals in fish sample

The concentration of different heavy metals Cd, and Ni were detected in different organs of fish collected from three different local fish markets. The mean concentration of different heavy metals studied in current investigation in different organs of different fish species are presented in tables below. The accumulation pattern of all the heavy metals in differential organs of all the studied fish species were significantly lower ($p > 0.05$) as compared to admissible concentration reported by WHO/FAO.

Cadmium Metal Results

The result of Cd concentration in three different organs of three different fish species are listed in Table 1 to Table 9. The mean Cd concentration ranged from (39.69 – 32.33ug/kg), (35.45 – 23.26ug/kg), (45.06 -35.37ug/kg) in gills (38.17- 28.99ug/kg), (30.07 – 21.74ug/kg), (43.61 – 33.92ug/kg) in muscles (35.56 – 26.12ug/kg), (31.32 – 18.87ug/kg), (41.01 – 31.05ug/kg) in liver of *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* respectively.

Nickle Metal Results

The result of Ni concentration in three different organs of three different fish species are listed in Table 10 to Table 18. The mean Ni concentration ranged from (44.93 – 52.86ug/kg), (45.65 – 52.86ug/kg), (52.30 -61.14ug/kg) in gills (43.48- 51.41ug/kg), (44.20– 54.62ug/kg), (50.86 – 59.69ug/kg) in muscles (40.48 – 48.81ug/kg), (41.56 – 48.81ug/kg), (48.21 – 57.08ug/kg) in liver of *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* respectively.

ANOVA Results

The collected data from heavy metal analysis were subjective to descriptive statistical analysis followed by one-way ANOVA $P < 0.05$ was considered indicating statistical significance.

Comparison of fish market

Comparison of Cd level in fish market

Results of descriptive statistics and ANOVA of Cd in fish market are summarized in table 3.19 and 3.20, respectively. Mean concentration of Cd did not vary significantly between the three markets. Cd level between

three market has following increasing order Bhaati Chowk < Shahdara < Do Moria Pull.

Comparison of Fish Species

Comparison of Ni level in fish market

Results of descriptive statistics and Anova of Ni in fish market are summarized in table 3.21 and 3.22, respectively. Mean concentration of Ni did not vary significantly between the three markets. Ni level between three market has following order Bhaati Chowk < Shahdara < Do Moria Pull.

Comparison of Cadmium level in fish species

Descriptive statistics and ANOVA results of cadmium in fish species are given in table 3.23, 3.24 respectively. Statistical analysis of Cd concentration is found to be non-significant among the three fish ($P < 0.05$). Decreasing order of cadmium concentration in fish species was as: *Cirrhina mrigala* > *Labeo rohita* > *Catla catla*.

The heavy metal concentration in different organs of Fish is shown its availability in a body of water can cause carcinogenic effects on aquatic biota and human health (US EPA) (Hadyait et al., 2019). The presence of these metals has many potential toxins. It has caused irregularities such as reduced development as well low growth rate and very low concentration of these metals like Cd can cause bone ossification (Mehana et al., 2020).

The present study was conduct to evaluate the concentration of heavy metals from thirty six (36) samples of three edible fish *Labeo rohita* (Rahu), *Catla catla* (Thela), *Cirrhina mrigala* (Mori) collected from three local fish market of Lahore (Bhatti Chowk, Shahdara, Do Mori Pull) (Hamid et al., 2016).

Heavy metals Cadmium (Cd), Nickel (Ni), were analyzed by the ICP-OES. Descriptive statistics and ANOVA results for cadmium and nickel in fish markets are summarized in table 3.1 to 3.26 respectively (Mehana et al., 2020). Fish markets have following decreasing order: Do Moria pull > Shahdara > Bhatti Chowk for Cadmium and Nickel. However, these results did not reveal any significant difference between different sampling sites (Green & Planchart, 2018).

Result for Nickel (Ni) metal concentration in three fish species are given in table 10 to table 18. The mean concentration for Ni ranged from 40.84ppb- 61.14ppb. Results for Cadmium (Cd) metal concentration in three fish species. The Cd concentration ranged from 18.87 ppb- 45.06ppb (Paschoalini & Bazzoli, 2021). The concentration of nickel also found highest in the gills as compared to other studied organs of the fish (Mehana et al., 2020). The lowest concentration was observed in the flesh of the fish. The same trend of bioaccumulation of

nickel was also observed highest in *C. catla* followed by *C. carpio* and *L. rohita*. Our study was like already reported literature by Gupta et al., 2009. The sampling site Do Moria pull showed the highest level of nickel as compared to other sites (Akoto et al., 2014).

Comparison of Nickel level in fish species

Descriptive statistics and ANOVA results of Nickel in fish species are given in table 3.25, 3.26 respectively. Statistical analysis of Ni concentration found a significant difference among the three fish ($P < 0.05$). Decreasing order of cadmium concentration in fish species was as: *Cirrhina mrigala* > *Catla catla* > *Labeo rohita*.

4. CONCLUSION

Current research has shown that the focus of heavy metals, cadmium, and nickel in the Gills, liver, and Flesh of three different species collected from different local fish markets of Lahore. The concentration of heavy metals did not exceed the permissible limits set by the studied heavy metals by regulations. Therefore, the fish of these areas did not pose serious threat to human health upon consumption. But it is suggested that precautionary measurements should be adopted so that the metals accumulation did not exceed permissible limits in fish for human consumption. Moreover, Fish has potential health benefits due to its high nutrient value. , however, it is essential that the levels of heavy metal do not exceed the permitted limits. The need for ongoing communication between health institutions, public organizations, and private businesses about the risks and benefits of fish consumption demonstrates both the need to examine the underlying assumptions that guide the development of public policy and the obligation to control the quality of fish and balance its benefits and risks.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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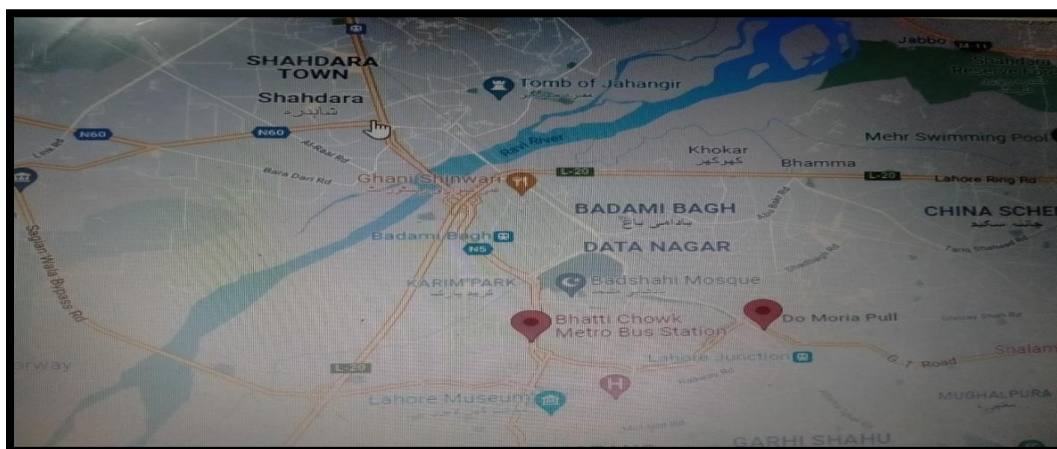


Figure 1. Map of Lahore showing sampling sites



Figure 2. ICP-OES instrument for metal detection



Figure 3. Sample preparation for metal analysis

Table 1. Concentration of Cd in gills of *Labeo rohita* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	30.91	34.63	38.62
2	34.04	33.04	41.101
3	30.41	30.51	38.74
4	33.971	34.37	40.321
MEAN	32.33275	33.1375	39.6955
SD	1.682242	1.632473	1.053134

Table 2. Concentration of Cd in gills of *Catla catla* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria pull
1	23.51	28.51	34.72
2	25.84	32.52	36.92
3	21.41	28.64	37.95
4	22.301	30.651	32.221
MEAN	23.26525	30.08025	35.45275
SD	1.662902	1.644547	2.20058

Table 3. Concentration of Cd in gills of *Cirrhinus mrigala* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	35.72	41.72	44.52
2	35.92	41.71	44.91
3	37.643	41.223	44.623
4	32.221	43.011	46.211
MEAN	35.376	41.916	45.066
SD	1.968984	0.663349	0.676337

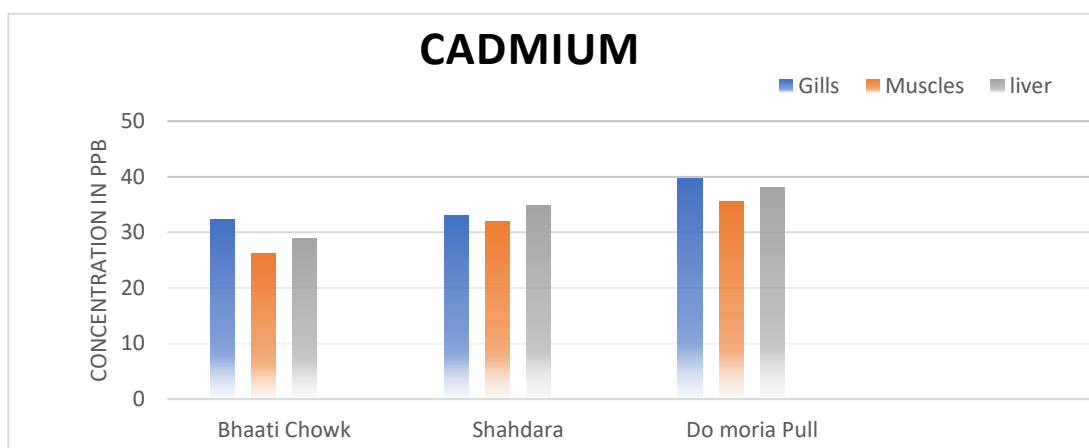


Figure 4. The accumulation of Cadmium (Cd) in organs (liver, gills, muscles) of fish sample *Labeo rohita* taken from different sampling site.

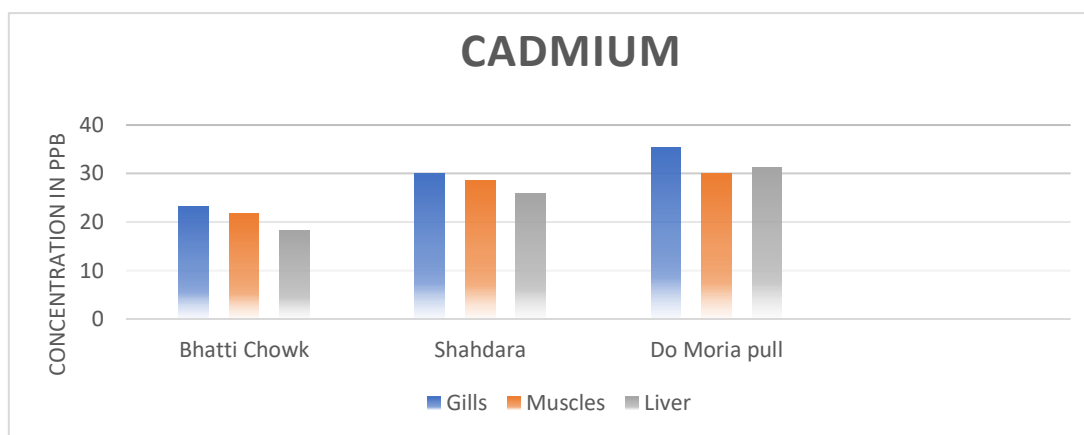


Figure 5. The accumulation of Cadmium (Cd) in organs (liver, gills, muscles) of fish sample *Catla catla* taken from different sampling site.

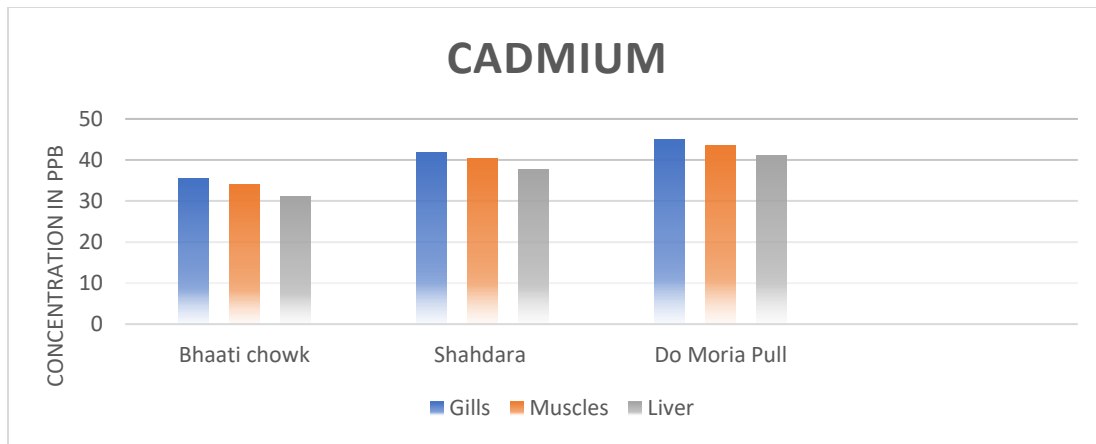


Figure 6. The accumulation of Cadmium (Cd) in organs (liver, gills, muscles) of fish sample *Cirrihinus mrigala* taken from different sampling site

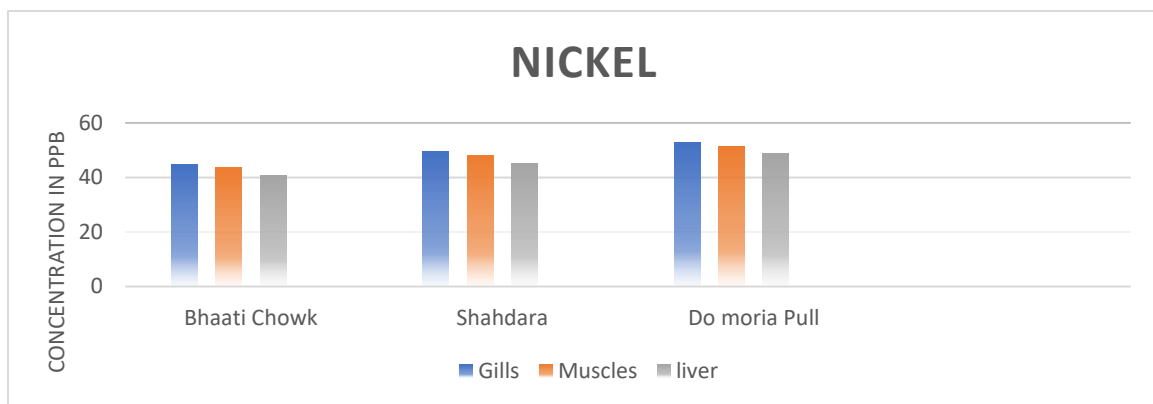


Figure 7. The accumulation of Nickel (Ni) in organs (liver, gills, muscles) of fish sample *Labeo rohita* taken from different sampling site.

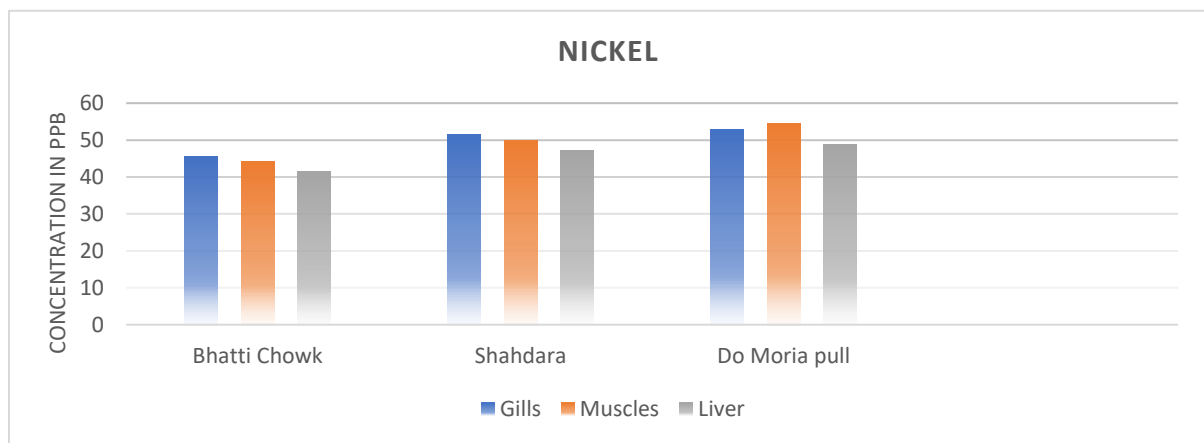


Figure 8. The accumulation of Nickel (Ni) in organs (liver, gills, muscles) of fish sample *Catla catla* taken from different sampling site.

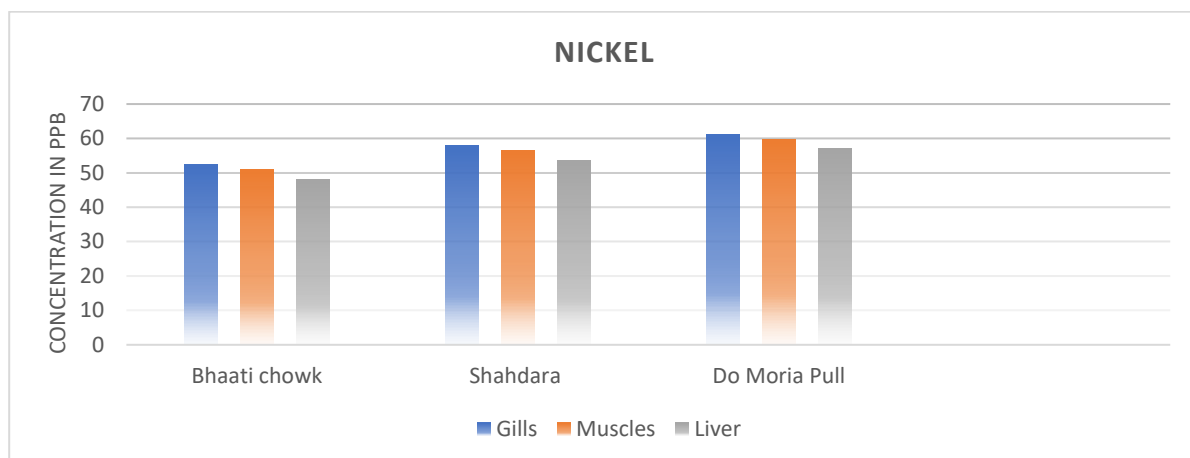


Figure 9. The accumulation of Nickel (Ni) in organs (liver, gills, muscles) of fish sample *Cirrihinus mrigala* taken from different sampling site.

Table 4. Concentration of Cd in muscles of *Labeo rohita* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	28.11	33.23	37.22
2	29.55	35.501	38.701
3	27.23	34.34	37.44
4	31.1	36.12	39.32
MEAN	28.9975	34.79775	38.17025
SD	1.469479	1.107958	0.87176

Table 5. Concentration of Cd in muscles of *Catla catla* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	22.11	27.11	29.33
2	23.44	30.12	30.12
3	20.11	27.34	31.1
4	21.3	29.65	29.76
MEAN	21.74	28.555	30.0775
SD	1.212167	1.342805	0.653237

Table 6. Concentration of Cd in muscles of *Cirrhinus mrigala* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	33.32	39.32	42.12
2	34.52	40.31	43.51
3	36.65	40.23	43.63
4	31.22	42.01	45.21
MEAN	33.9275	40.4675	43.6175
SD	1.96613	0.971761	1.094335

Table 7. Concentration of Cd in Liver of *Labeo rohita* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	25.51	31.6	35.02
2	25.91	34.141	35.801
3	25.45	29.78	33.45
4	27.64	32.69	37.98
MEAN	26.1275	32.05275	35.56275
SD	0.890965	1.591952	1.632341

Table 8. Concentration of Cd in Liver of *Catla catla* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	19.51	25.48	31.12
2	19.8	28.76	31.62
3	18.33	22.78	32.66
4	17.84	26.22	29.88
MEAN	18.87	25.81	31.32
SD	0.810401	2.130751	0.9999

Table 9. Concentration of Cd in Liver of *Cirrhinus mrigala* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	30.72	37.69	39.92
2	30.88	38.95	40.61
3	34.87	35.67	39.64
4	27.76	38.58	43.87
MEAN	31.0575	37.7225	41.01
SD	2.527552	1.270421	1.688535

Table 10. Concentration of Ni in Gills of *Labeo rohita* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	45.61	49.83	53.03
2	46.93	47.83	50.93
3	44.863	50.313	53.513
4	42.331	50.003	53.993
MEAN	44.9335	49.494	52.8665
SD	1.674936	0.97617	1.168731

Table 11. Concentration of Ni in Gills of *Catla catla* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	46.83	50.89	53.03
2	46.72	50.4	50.93
3	47.753	51.193	53.513
4	41.301	53.341	53.993
MEAN	45.651	51.456	52.8665
SD	2.54331	1.124487	1.168731

Table 12. Concentration of Ni in Gills of *Cirrhinus mrigala* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	53.51	60.62	63.52
2	51.63	55.62	57.82
3	49.893	57.423	60.823
4	54.201	58.101	62.401
MEAN	52.3085	57.941	61.141
SD	1.682272	1.792903	2.143444

Table 13. Concentration of Ni in Muscles of *Labeo rohita* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	43.21	47.43	50.63
2	45.53	46.43	49.53
3	43.87	49.32	52.52
4	41.33	49.002	52.992
MEAN	43.485	48.0455	51.418
SD	1.504153	1.175577	1.403332

Table 14. Concentration of Ni in Muscles of *Catla catla* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	44.43	48.49	55.1
2	45.32	49	53.2
3	46.76	50.2	53.1
4	40.3	52.34	57.1
MEAN	44.2025	50.0075	54.625
SD	2.401608	1.482858	1.636116

Table 15. Concentration of Ni in Muscles of *Cirrhinus mrigala* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	51.11	58.22	61.12
2	50.23	54.22	56.42
3	48.9	56.43	59.83
4	53.2	57.1	61.4
MEAN	50.86	56.4925	59.6925
SD	1.563378	1.459578	1.979992

Table 16. Concentration of Ni in Liver of *Labeo rohita* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	40.31	45.1	48.43
2	42.09	44.53	46.63
3	41.54	46.42	48.53
4	39.43	45.012	51.652
MEAN	40.8425	45.2655	48.8105
SD	1.039408	0.700978	1.806389

Table 17. Concentration of Ni in Liver of *Catla catla* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	41.53	46.16	48.43
2	41.88	47.1	46.63
3	44.43	47.3	48.53
4	38.4	48.35	51.652
MEAN	41.56	47.2275	48.8105
SD	2.140432	0.77799	1.806389

Table 18. Concentration of Ni in Liver of *Cirrhinus mrigala* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	48.21	55.89	58.92
2	46.79	52.32	53.52
3	46.57	53.53	55.84
4	51.3	53.11	60.06
MEAN	48.2175	53.7125	57.085
SD	1.887715	1.33012	2.572795

Table 19. Descriptive statistics of Cd in fish market

Sampling Sites	N	Mean	S. D	Minimum	Maximum
Bhaati Chowk	12	27.966	5.695226	17.84	37.643
Shahdara	12	33.83769	5.310839	22.78	43.011
Do Moria Pull	12	37.77469	5.03452	29.33	46.211
Total	36	33.1928	5.346862	23.31667	42.28833

N= Number of fish

Table 20. ANOVA of Cd in fish market

	Sum of squares	Degree of freedom	Mean square	F	Significant
Between groups	1754	2	877.1	29.75	0.7669
Within groups	3096	105	29.48		
Total	4850	107			

Significant $p < 0.05$

Table 21. Descriptive statistics of Ni in fish market

Sampling Sites	N	Mean	S. D	Minimum	Maximum
Bhaati Chowk	12	45.7845	4.174639	38.4	54.201
Shahdara	12	51.07133	4.202806	44.53	60.62
Do Moria Pull	12	54.14617	4.53689	46.63	63.52
Total	36	50.334	4.304778	43.18667	59.447

N= Number of fish

Table 22. ANOVA of Ni in fish market

	Sum of squares	Degree of freedom	Mean square	F Value	Significant
Between groups	1288	2	643.9	33.73	0.0778
Within groups	2004	105	19.09		
Total	3292	107			

Significant $p < 0.05$

Table 23. Descriptive statistics for Cadmium in fish species

Fish species	N	Mean	S. D	Minimum	Maximum
<i>Labeo rohita</i>	12	33.4256	4.24846	26.12	39.69
<i>Catla catla</i>	12	27.2389	5.24444	18.87	35.45
<i>Cirrhina mrigala</i>	12	38.9011	4.69510	31.05	45.06
Total	36	33.1885	6.66094	18.87	45.06

N= Number of fish

Table 24. ANOVA of Cadmium in fish species

	Sum of squares	Degree of freedom	Mean square	F Value	Significant
Between groups	612.79	2	306.39	13.59	0.1876
Within groups	540.78	24	22.53		
Total	1153.57	26			

Significant $p < 0.05$

Table 25. Descriptive statistics for Nickel in fish species

Fish species	N	Mean	S. D	Minimum	Maximum
<i>Labeo rohita</i>	12	47.23	3.8970	40.84	52.86
<i>Catla catla</i>	12	48.48	4.2415	41.56	54.62
<i>Cirrhina mrigala</i>	12	55.26	4.8783	48.21	61.14
Total	36	50.33	5.3643	40.84	61.14

N= Number of fish

Table 26. ANOVA of Nickel in fish species

	Sum of squares	Degree of freedom	Mean square	F Value	Significant
Between groups	336.33	2	168.16	9.800	0.001
Within groups	411.85	24	17.16		
Total	748.18	26			

Significant $p < 0.05$