Supplementary material

S.2.6. Data analysis using multiple pollution and risk assessment approaches

2.6.1. Coefficient of condition (K)

The coefficient of condition (K) is a measure used to assess the general health and well-being of fish, typically reflecting their nutritional status and growth trends.

A species' K value of >1 indicates that they are in perfect condition, whereas a value of <1 indicates that the fish's health is not good [50].

2.6.2. Metal pollution index (MPI)

Using the metal pollution index (MPI), the relative severity of total metal deposition in the muscles of fish was ascertained.

MPI of 2 to 3 suggests a moderate level of pollution, 1 to 2 indicates a mild level of pollution, and less than 1 shows negligible level of pollution impact [122].

2.6.3. Bioconcentration factor (BCF)

The net effect of a material entering the biota through water and being absorbed, distributed, and eliminated is known as the bioconcentration factor [82].

BCF >1 is an indication of efficient uptake of a contaminant by the organism. A BCF<1 fewer uptake of the contaminant [123].

S2.7. Health Risk Assessment

Interpretation of Parameters in Equation (5): Average Daily Dose (ADD)

- **C (bioaccessible):** Bioaccessible concentration of metal in fish muscle (mg/kg), estimated using *in vitro* digestion methods.
- **IR (Ingestion Rate):** The amount of fish consumed per day:
    - Fisherfolk adults: 0.10 mg/day
    - Fisherfolk children: 0.05 mg/day
    - General population adults: 0.01 mg/day
    - General population children: 0.005 mg/day

- **EF (Exposure Frequency):** Number of exposure days per year (365 days/year).
- **ED (Exposure Duration):**
    - Adults: 70 years
    - Children: 10 years
- **BW (Body Weight):**

Adults: 73 kg

Children: 32.7 kg

- **AT (Averaging Time):**

- For non-carcinogenic risk: ED × 365 days
    - Adults: 25,550 days
    - Children: 3,650 days [124-127]

Interpretation of Equation (6): Hazard Quotient (HQ)

- **HQ (Hazard Quotient):** Ratio of estimated metal exposure to its reference dose.
- **RfD (Reference Dose):** Acceptable daily oral intake of metal (mg/kg/day). Values used [128]:
    - Zn: 0.3
    - Cd: 0.001
    - Pb: 0.006
    - Ni: 0.02
    - Al: 1.00
    - As: 0.0003
    - Co: 0.0003
    - Cr: 0.003
    - Cu: 0.04
    - Mn: 0.14
    - Se: 0.005

- $HQ \leq 1 \rightarrow$ No significant health risk
- $HQ > 1 \rightarrow$ Potential non-carcinogenic health effects

Interpretation of Equation (7): Hazard Index (HI)

- **HI (Hazard Index):** Sum of HQs for all metals assessed for a specific population group.

- $HI \leq 1 \rightarrow$ Cumulative exposure is within safe limits
- $HI > 1 \rightarrow$ Potential health concern due to combined metal exposure [129-131].

Interpretation of Equation (8): Carcinogenic Risk (CR)

- **CR (Carcinogenic Risk):** Probability of developing cancer over a lifetime due to exposure to a carcinogenic metal.
- **CSFo (Cancer Slope Factor oral):** Indicates the risk per mg/kg/day of intake. Values used [132]:
    - Cd: 0.38
    - Pb: 0.0085
    - Ni: 1.7
    - As: 1.5
    - Cr: 0.5

- $CR > 10^{-4} \rightarrow$ Unacceptable cancer risk
- $10^{-6} < CR \leq 10^{-4} \rightarrow$ Acceptable risk
- $CR \leq 10^{-6} \rightarrow$ Negligible risk

Table S1.Common and scientific names, feeding nature and habitat of the examined fish species from Rawal Lake

Common name	Scientific name	Feeding nature and behavior	Habitat	References
Silver carp	*Hypophthalmichthys molitrix*	Herbivorous, Surface feeder	Surface layer	[133]
Mozambique Tilapia	*Oreochromis mossambicus*	Omnivorous	shallow water upto 30cm deep	[134]
Mori/Murakhi	*Cirrhinus mrigal*	Omnivorous/Detritivorous, Bottom feeder	Bottom layer	[135]
Thala	*Catla catla*	Planktivorous, Surface feeder	Surface layer	[136]
Grass Carp	*Ctenopharyngodon idella*	Herbivorous, Surface feeder	Surface layer	[133]
Common carp	*Cyprinus Carpio*	Omnivorous	Littoral zone in spring, Deep water in winter	[137]

Table S2.The geographic coordinates of the sampling sites and surrounding catchment areas

Water Sampling Site	Latitude	Longitude
1	33.6972	73.1253
2	33.7167	73.1481
3	33.7017	73.1233
4	33.7108	73.1316
5	33.7106	73.1260
Catchment area	**Latitude**	**Longitude**
Islamabad club	33.7003	73.1064
Bani Gala	33.7124	73.1584
Lake view park	33.7153	73.1305
Malpur	33.7275	73.1495

Table S3.Physicochemical properties of water samples collected from Rawal Lake

Sampling sites	pH	EC(uSC/cm)	TDS(mg/l)	Turbidity(NTU)	Hardness(mg/l)
WHO guidelines(2002)	**6.5-8.5**	**<1000**	**<500**	**<5**	**<500**
Site 1	8.55±0.31	398±10.64	224±7.72	0.94±0.01	156±17.18
Site 2	8.96±0.44	391±31.54	202±18.07	0.78±0.02	173±17.69
Site 3	9.84±0.15	395±16.02	210±11.35	1.02±0.07	136±2.41
Site 4	7.69±0.32	427±17.91	231±16.56	0.92±0.03	186±7.99
Site 5	8.28±0.93	389±27.72	207±13.70	0.83±0.01	131±8.84

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96 **Table S4**. The concentrations of heavy metals in water samples collected from five different sites of Rawal Lake (mg/l).

Water sample	Zn	Cd	Pb	Ni	Al	As	Ca	Co	Cr	Cu	Mn	Se
Site 1	1.36±0.06^{b}	0.003±0.001^{bc}	0.54±0.04^{a}	0.24±0.04^{ab}	10.22±0.10^{b}	0.59±0.01^{a}	43.65±0.05^{b}	0.95±0.02^{b}	0.42±0.01^{c}	0.28±0.01^{d}	0.75±0.01^{d}	0.03±0.01^{c}
Site 2	2.75±0.05^{a}	0.007±0.001^{a}	0.73±0.01^{a}	0.29±0.01^{b}	12.48±0.08^{a}	2.35±0.05^{b}	65.2±0.10^{a}	2.48±0.02^{a}	0.63±0.01^{a}	1.83±0.03^{a}	6.99±0.04^{a}	0.87±0.01^{a}
Site 3	1.44±0.04^{b}	0.002±0.0002^{c}	0.44±0.02^{a}	0.24±0.01^{ab}	6.65±0.05^{d}	1.48±0.03^{c}	28.32±0.02^{e}	0.63±0.01^{d}	0.05±0.01^{b}	0.62±0.01^{c}	2.76±0.01^{c}	0.65±0.01^{a}
Site 4	2.52±0.02^{a}	0.005±0.001^{ab}	0.62±0.02^{a}	0.23±0.01^{a}	4.75±0.05^{e}	0.07±0.01^{d}	41.72±0.02^{c}	0.03±0.01^{e}	0.58±0.01^{d}	0.08±0.01^{e}	3.84±0.02^{b}	0.14±0.01^{b}
Site 5	2.54±0.04^{a}	0.006±0.001^{a}	0.45±0.01^{a}	0.28±0.01^{ab}	8.73±0.03^{c}	0.86±0.01^{e}	32.68±0.03^{d}	0.82±0.01^{c}	0.06±0.01^{c}	0.91±0.01^{b}	0.21±0.01^{e}	0.09±0.01^{c}
Average	2.12±0.67	0.0046±0.002	0.56±0.122	0.26±0.027	8.57±3.013	1.07±0.877	42.31±14.27	0.98±0.91	0.35±0.28	0.74±0.69	2.91±2.72	0.36±0.38
WHO [138]	3.00	3.00	0.01	0.07	0.20	0.01	100.00	0.04	0.05	2.00	0.10	0.04
PakEPA [46]	5.00	0.01	0.05	0.02	N/A	N/A	200.00	N/A	0.05	2.00	0.50	N/A
USEPA [139]	5.00	5.00	0.01	0.70	0.05-0.02	0.01	N/A	N/A	0.10	1.30	5.00	0.05

97 Values are presented as mean ± SD (n = 3). Means with different lowercase letters within a column are significantly different ($p < 0.05$, Tukey
98 Honestly Significant Difference test).

Table S5.Weight, complete length and coefficient of condition (K) of different fish species from Rawal Lake.

Fish specie	Weight(g)	Complete length(mm)	Coefficient of condition(gmm^{-3})
H.molitrix	475±107.07^{b}	340.36±28.74^{b}	1.20±0.03^{bc}
O.mossambicus	183.3±28.87^{e}	207.77±16.13^{e}	2.08±0.16^{a}
C.mrigal	600±50^{a}	378.9±9.57^{a}	1.11±0.13^{c}
C.catla	300±35.36^{d}	271.78±5.39^{d}	1.55±0.08^{b}
C.idella	350±21.21^{c}	284.48±3.59^{cd}	1.56±0.06^{b}
C.carpio	350±7.07^{c}	294.64±7.18^{c}	1.42±0.08^{bc}

Values are presented as mean ± SD (n = 3). Means with different lowercase letters within a column are significantly different ($p < 0.05$, Tukey Honestly Significant Difference test).

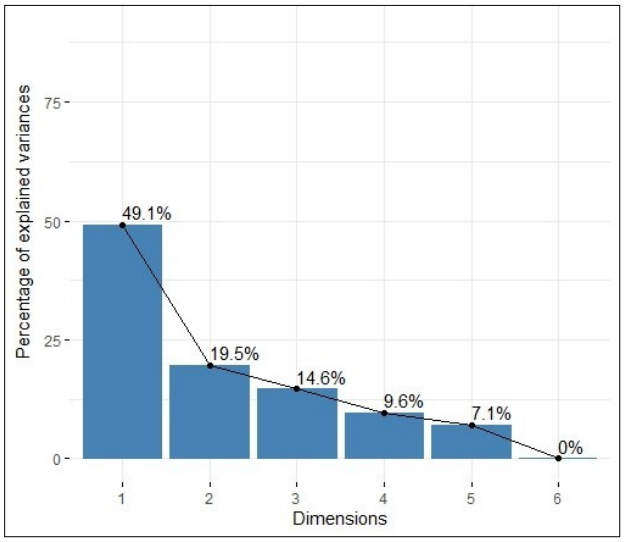
Table S6. Bioconcentration factor (BCF) of heavy metals in examined fish species.

Fish Species	Zn	Cd	Pb	Ni	Al	As	Ca	Co	Cr	Cu	Mn	Se
H.molitrix	2.34±0.09^{d}	40.58±0.32^{e}	0.701±0.03^{c}	0.20±0.02^{d}	0.41±0.03^{d}	0.07±0.01^{e}	0.82±0.02^{a}	0.09±0.01^{a}	0.16±0.02^{d}	0.11±0.01^{a}	0.03±0.01^{a}	0.14±0.01^{d}
O.mossambicus	1.94±0.03^{e}	47.81±0.10^{d}	0.08±0.02^{e}	0.21±0.02^{d}	0.59±0.02^{c}	0.07±0.01^{e}	0.28±0.01^{d}	0.08±0.01^{a}	0.11±0.01^{e}	0.05±0.01^{c}	0.02±0.01^{ab}	0.25±0.01^{a}
C.mrigal	3.45±0.04^{b}	105.00±0.13^{a}	4.09±0.04^{a}	2.62±0.02^{a}	1.18±0.02^{a}	1.83±0.03^{a}	0.60±0.02^{c}	0.02±0.01b^{c}	3.68±0.02^{a}	0.01±0.01^{d}	0.01±0.01^{b}	0.19±0.01^{b}
C.catla	2.63±0.03^{c}	84.78±0.20^{b}	3.69±0.02^{b}	0.12±0.01^{e}	0.11±0.01^{e}	0.14±0.01^{d}	0.23±0.01^{e}	0.01±0.01^{c}	0.23±0.01^{c}	0.08±0.01^{b}	0.01±0.01^{b}	0.17±0.01^{bc}
C.idella	1.67±0.02^{f}	15.94±0.08^{f}	0.10±0.02^{e}	0.27±0.01^{c}	0.62±0.02^{c}	0.35±0.01^{c}	0.24±0.01^{e}	0.02±0.01^{bc}	2.04±0.01^{b}	0.06±0.01^{bc}	0.01±0.01^{b}	0.17±0.01^{bc}
C.carpio	4.02±0.03^{a}	49.27±0.15^{c}	0.33±0.02^{d}	2.15±0.04^{b}	0.82±0.02^{b}	0.97±0.02^{b}	0.73±0.01^{b}	0.04±0.01^{b}	0.26±0.01^{c}	0.01±0.01^{d}	0.005±0.01^{ab}	0.16±0.01^{cd}

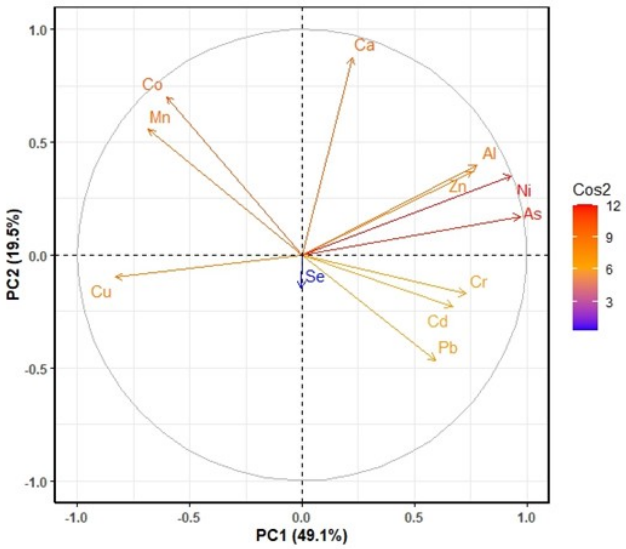
Values are presented as mean ± SD (n = 3). Means with different lowercase letters within a column are significantly different ($p < 0.05$, Tukey Honestly Significant Difference test).

Table S7. Principal component analysis (PCA) factor score for heavy metals concentrations in different fish species of Rawal Lake.

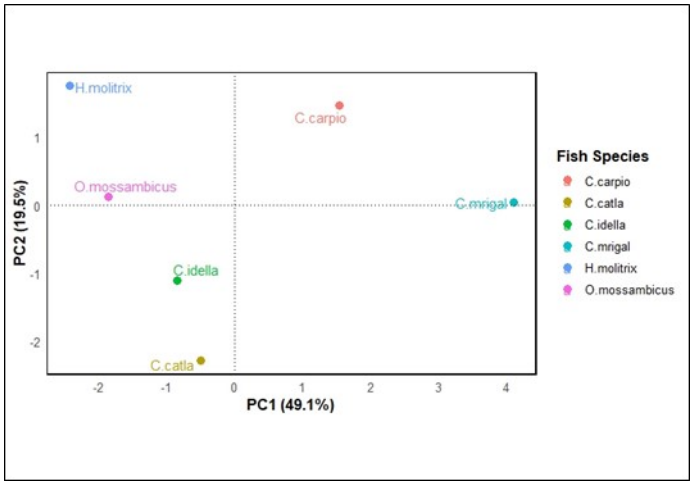
Variables	PC1	PC2
Zn	0.31	0.24
Cd	0.28	-0.15
Pb	0.24	-0.30
Ni	0.38	0.23
Al	0.32	0.26
As	0.40	0.11
Ca	0.09	0.57
Co	-0.25	0.46
Cr	0.30	-0.11
Cu	-0.34	-0.06
Mn	-0.28	0.36
Se	0.00	-0.10
Eigen value	5.89	2.35
Standard deviation	2.43	1.53
Proportion of variance	0.49	0.20
Cumulative proportion	0.49	0.69



(a). Scree plot



(b). Loading plot



c) Score plot

Figure S1 (**a)** Scree plot **(b)** Loading plot **(c)** Score plot of PCA of heavy metals and the examined fish species

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Fish Species	Zn	Cd	Pb	Ni	Al	As	Ca	Co	Cr	Cu	Mn	Se
H.molitrix	32.90±1.21^{c}	75.00±2.36^{a}	34.62±0.96^{c}	47.00±4.08^{b}	66.56±5.00^{a}	75.00±1.59^{a}	70.22±5.75^{ab}	55.56±3.79^{b}	35.09±2.07^{b}	50.63±8.02^{b}	41.09±3.01^{ab}	80.00±6.06^{a}
O.mossambicus	24.23±1.53^{c}	36.36±2.87^{b}	57.45±2.21^{b}	36.36±0.95^{c}	73.01±1.50^{a}	28.57±0.55^{b}	64.73±1.08^{bc}	75.00±2.71^{a}	25.00±3.04^{c}	52.63±2.54^{c}	17.86±0.61^{d}	33.07±1.69^{c}
C.mrigal	55.66±1.70^{b}	74.53±1.50^{a}	87.40±4.47^{a}	50.75±1.00^{b}	41.06±6.06^{b}	30.10±3.73^{b}	34.93±2.65^{d}	30.00±4.07^{c}	20.31±1.43^{cd}	40.00±1.10^{cd}	33.33±2.34^{c}	86.95±10.48^{a}
C.catla	51.61±2.98^{b}	51.28±6.71^{b}	52.19±6.26^{b}	66.67±3.06^{a}	28.45±2.02^{c}	20.00±1.45^{a}	54.30±1.43^{c}	45.45±3.76^{b}	50.00±1.45^{a}	34.48±2.18^{a}	33.33±1.91^{c}	33.79±2.68^{c}
C.idella	69.77±0.96^{a}	40.91±2.78^{b}	35.29±1.05^{c}	28.57±3.66^{d}	67.07±1.72^{a}	67.56±5.60^{b}	74.56±3.58^{a}	50.20±4.46^{b}	18.30±2.45^{d}	23.26±2.67^{d}	38.89±2.35^{bc}	80.65b±1.07^{a}
C.carpio	52.29±9.15^{b}	66.18±10.18^{a}	40.36±5.70^{c}	47.27±3.04^{b}	46.22±1.10^{b}	61.04±12.50^{a}	73.80±5.23^{ab}	50.00±4.68^{b}	33.33±2.70^{b}	45.45±3.39^{b}	50.00±7.42^{a}	51.91±4.58^{b}

108 **Table S8.** Bioaccessibility (%) of heavy metals in muscles of different fish species.

109 Values are presented as mean ± SD (n = 3). Means with different lowercase letters within a column are significantly different ($p < 0.05$, Tukey
110 Honestly Significant Difference test).

Table S9. Average daily dose (ADD) of heavy metals content (mg/kg/day) through Rawal lake fish consumption by the human population based on bioaccessible fraction

Fish species	Population	Age group	Zn	Cd	Pb	Ni	Al	As	Ca	Co	Cr	Cu	Mn	Se
	Fisherfolk	Children	2.5E-03	2.1E-04	2.1E-04	3.6E-05	3.6E-03	9.2E-05	3.7E-02	7.6E-06	3.1E-05	6.1E-05	4.6E-05	6.1E-05
H.molitrix		Adult	2.2E-03	1.9E-04	1.8E-04	3.2E-05	3.2E-03	8.2E-05	3.4E-02	6.8E-06	2.7E-05	5.5E-05	4.1E-05	5.5E-05
	General	Children	2.5E-04	2.1E-05	2.1E-05	3.6E-06	3.6E-04	9.2E-06	3.7E-03	7.6E-07	3.1E-06	6.1E-06	4.6E-06	6.1E-06
		Adult	2.2E-04	1.9E-05	1.8E-05	3.2E-06	3.2E-04	8.2E-06	3.4E-03	6.8E-07	2.7E-06	5.5E-06	4.1E-06	5.5E-06
	Fisherfolk	Children	1.5E-03	1.2E-04	4.1E-05	3.1E-05	5.6E-03	3.1E-05	1.2E-02	9.2E-05	1.5E-05	3.1E-05	8.6E-05	4.6E-05
O.mossambicus		Adult	1.4E-03	1.1E-04	3.7E-05	2.7E-05	5.0E-03	2.7E-05	1.0E-02	8.2E-05	1.4E-05	2.7E-05	7.7E-05	4.1E-05
	General	Children	1.5E-04	1.2E-04	4.1E-06	3.1E-06	5.6E-04	3.1E-06	1.2E-03	9.2E-06	1.5E-06	3.1E-06	8.6E-06	4.6E-06
		Adult	1.4E-04	1.1E-05	3.7E-06	2.7E-06	5.0E-04	2.7E-06	1.0E-03	8.2E-06	1.4E-06	2.7E-06	7.7E-06	4.1E-06
	Fisherfolk	Children	6.2E-03	5.5E-04	3.0E-03	5.2E-04	6.3E-03	9.0E-04	1.3E-02	9.2E-06	4.0E-04	6.1E-06	2.3E-05	9.2E-05
C.mrigal		Adult	5.6E-03	4.9E-04	2.7E-03	4.7E-04	5.7E-03	8.1E-04	1.2E-02	8.2E-06	3.6E-04	5.5E-06	2.1E-05	8.2E-05
	General	Children	6.2E-04	5.5E-05	3.0E-04	5.2E-05	6.3E-04	9.0E-05	1.3E-03	9.2E-07	4.0E-05	6.1E-07	2.3E-06	9.2E-06
		Adult	5.6E-04	4.9E-05	2.7E-04	4.7E-05	5.7E-04	8.1E-05	1.2E-03	8.2E-07	3.6E-05	5.5E-07	2.1E-06	8.2E-06
	Fisherfolk	Children	4.4E-03	3.1E-04	1.6E-03	3.1E-05	4.1E-04	4.6E-05	8.1E-03	7.6E-06	6.1E-05	3.1E-05	1.8E-05	3.1E-05
C.catla		Adult	3.9E-03	2.7E-04	1.5E-03	2.7E-05	3.7E-04	4.1E-05	7.3E-03	6.8E-06	5.5E-05	2.7E-05	1.6E-05	2.7E-05
	General	Children	4.4E-04	3.1E-05	1.6E-04	3.1E-06	4.1E-05	4.6E-06	8.1E-04	7.6E-07	6.1E-06	3.1E-06	1.8E-06	3.1E-06
		Adult	3.9E-04	2.7E-05	1.5E-04	2.7E-06	3.7E-05	4.1E-06	7.3E-04	6.8E-07	5.5E-06	2.7E-06	1.6E-06	2.7E-06
	Fisherfolk	Children	3.8E-03	4.6E-05	3.1E-05	2.3E-05	5.5E-03	3.8E-04	1.2E-02	1.5E-05	2.0E-04	1.5E-05	2.8E-05	7.6E-05
C.idella		Adult	3.4E-03	4.1E-05	2.7E-05	2.1E-05	4.9E-03	3.4E-04	1.0E-02	1.4E-05	1.8E-04	1.4E-05	2.5E-05	6.8E-05
	General	Children	3.8E-04	4.6E-06	3.1E-06	2.3E-06	5.5E-04	3.8E-05	1.2E-03	1.5E-06	2.0E-05	1.5E-06	2.8E-06	7.6E-06
		Adult	3.4E-04	4.1E-06	2.7E-06	2.1E-06	4.9E-04	3.4E-05	1.0E-03	1.4E-06	1.8E-05	1.4E-06	2.5E-06	6.8E-06
	Fisherfolk	Children	6.8E-03	2.3E-04	1.1E-04	4.0E-04	4.9E-03	9.6E-04	3.5E-02	3.1E-05	4.6E-05	7.6E-06	2.4E-05	4.6E-05
C.carpio		Adult	6.1E-03	2.1E-04	1.0E-04	3.6E-04	4.4E-03	8.6E-04	3.1E-02	2.7E-05	4.1E-05	6.8E-06	2.2E-05	4.1E-05
	General	Children	6.8E-04	2.3E-05	1.1E-05	4.0E-05	4.9E-04	9.6E-05	3.5E-03	3.1E-06	4.6E-06	7.6E-07	2.4E-06	4.6E-06
		Adult	6.1E-04	2.1E-05	1.0E-05	3.6E-05	4.4E-04	8.6E-05	3.1E-03	2.7E-06	4.1E-06	6.8E-07	2.2E-06	4.1E-06