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Original Article

Assessment of water quality and targeted accumulation of some heavy metals in different organs of fresh water fish *Ompok bimaculatus* in riverine system of Punjab, Pakistan

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ABSTRACT

Heavy metals are metals that have high-density and are non-biodegradable. Among all pollutants, heavy metal pollution is the most serious. *Ompok bimaculatus* is a freshwater fish that belongs to cat family. **Objective:** To estimate concentration of six heavy metals (Copper, Ferric, Cadmium, Chromium, Nickel and Lead) in water, sediments and some organs (gills, fins and muscles) of fish collected from river Ravi. **Methods:** The accumulation level was recorded through atomic absorption spectrophotometer whereas, physiochemical parameters were also studied through testing water quality. **Results:** The heavy metal analysis indicated that the level of Fe was maximum in water and sediments followed by Ni and Pb. Other metals, Cu and Cr were also above permissible standards for drinking water. Further metal concentration was lower in bank water of river than in the main stream. The gills have higher metal accumulation level which is most likely due to direct contact with water followed by fins and muscles. The pattern of accumulation was Cadmium < Chromium < Nickel < Cupper < Lead < Ferric in fish organs. Higher concentrations of ALP and ASP than reference value which showed that the metal pollutants had started damaging the fish organs. **Conclusions:** In conclusion, the accumulation of all studied metals was higher in water and sediments than WHO permission standards. Same was true in the case of studied fish organs. Further, metals also fluctuate the hematological parameters of *O. bimaculatus*.

INTRODUCTION

Ompok bimaculatus is a catfish. Its status is an endangered fish species [1]. This fish is present in south Asian countries. This fish is highly nutritious and delicious, because its meat is soft and contain high concentration of lipoprotein [2]. The population of *O bimaculatus* reduced dramatically in recent years [3, 4]. The metals which have high-density and non-biodegradable metallic elements and have long lasting effect have serious problem. Heavy metals mixed with water through public sewage, agricultural waste and industrial waste. These effluents leached into groundwater or release in riverine water through runoffs water [5]. Metals accumulated in aquatic environment and reach to the aquatic biota [6]. The increased concentration of heavy metals are endangered for some species and cause their extinction of some aquatic species [7]. Metals accumulate in different organs through gills and feeding contents taken up by fish [8]. Heavy metals cause cancer and mutation and also cause disease [9]. For example, mis-regulation of iron metabolism leads to neurodegenerative disease [10]. Organs which can be most commonly affected by over dose of iron (hemochromatosis) are the liver, heart, and endocrine glands [11]. Some specific islet beta cells of pancreas cause diabetes [12]. Nickel targets kidney, lungs, liver and cause multiple toxic effects after entering the body. Nickel is also a harmful carcinogenic substance.

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Some other diseases are also caused by nickel including apoptosis, breast cancer, infertility, lung cancer and fibrosis [13]. An exposure to high amount of lead in short time can cause lead poising. The accumulation of lead effects brain firstly. Symptoms of excess may include irritability, constipation and memory problems. Excess accumulation of heavy metals may also lead to infertility [14]. Wilson's disease caused by accumulation of copper [15]. Accumulation of copper also cause some liver disease. Accumulation of cadmium increased blood glucose level and cause diabetes. Cd had direct cytotoxic effects on pancreas by decreasing insulin levels [16]. Accumulation of excess chromium causes chromium toxicity. The symptoms of Chromium Toxicity include ulcer, renal failure, vomiting and nausea. The aim and objective of present study was to determine toxicological effect of heavy metals on sediments, water quality and evaluate the accumulation in different organs of fresh water fish *O. bimaculatus*.

METHODS

Water and sediment samples were obtained at several locations along the Ravi River (main stream and river bank). Water samples were taken using a Van Dorn Bottle Sampler, while sediment samples were gathered using a steel pipe 3 feet deep. From each location, three *O. bimaculatus* specimens were collected. On the spot, the fixation of dissolved oxygen (D.O) in water was measured. Standard procedures were used to measure pH, temperature, total solids (T.S), and TDS. Water samples were collected in plastic bottles and acidified with laboratory grade Nitric acid (HNO_3) at pH2. Sediment and fish samples were also stored in zip lock plastic bags and transported in an icebox. FAO Technical Paper No. 212 was used to arrange the sub samples. Fish was partially melted for metal analysis, and each specimen (fish) was dissected with a stainless steel blade. The investigation used separated samples of 2-5 g of kidney, muscles, and gills. At 100 °C, fish samples were dissolved in pure Nitric acid and per chloric acid for gills 4:1 till the solution was transparent. Deionized distilled water was used to create the needed solution. To obtain a sediment sample, 4-5 g of sediment was dried in an oven at 100 °C for 48 hours. The dried sediment samples were passed through a conventional screen mesh to eliminate bigger particles. In addition, 1 g of each sediment sample was transferred to a 100 mL quartz tube and placed on a heated plate before being digested in HCl (1:3 v/v) and concentrated HNO_3. The quartz tubes had been cooled. The Atomic Absorption Spectrophotometer was used to examine the buildup of Cadmium, Copper, Lead, Chromium, Ferric, and Nickel in samples. The results were given in mg/L and µg/g. This index is widely used for calculation of actual values of heavy metal pollution with respect to the approved limit for standard discharge of environmental pollutant into water [17]. The pollution index of heavy metal individual was estimated by the following formula:

$$PI = \frac{\text{Measured concentration of individual heavy metal}}{\text{Standard permisible concentration of heavy metal}}$$

According to Estiarte *et al*. and Michell *et al*., the actions of the ACP and ALP enzymes were observed. This method used 0.75 ml of pH-5 CH_3COONa buffer as the reaction mixture [18, 19]. Then 0.25 ml of 5 mM p. nitro-phenyl phosphate was added. This mixture served as the substrate, and 0.5 cc of enzyme was added once more. The entire mixture was reduced to 1 ml in volume. the reaction mixture was incubated at 37°C for 30 minutes. The substrate mixture for ALP contained 0.5 ml of buffered glycine with a pH of 7.8, 0.2 ml of 10 mM $MgCl_2$, and 0.25 ml of 5 mM p.nitro-phenyl phosphate. Finally, the reaction mixture was diluted to 1 ml and incubated at 37°C for 30 minutes after adding approximately 0.05 ml of the enzyme. The activity of both enzymes was monitored using a Synergy Multi-Mode Microplate Reader. The activity was calculated using the coloration (measurement of yellow colour) of p. nitro-phenol. Tukey's pairwise comparison test was used to compare the average and standard deviation of three replicates. Heavy metal concentrations were calculated using the standard curve approach in Microsoft Excel (V.10).

RESULTS

The current study was planned to record heavy metals concentration in water, sediments and different organs (gills, Muscles, Fins) of fish. Water physicochemical are listed in Table 1.

Table 1: Physiochemical parameters of water sampled in raver Ravi

Sampling season	Winter
Temperature(°C)	15.29±0.24
pH	8.47±0.55
Hardness (mg/L)	149.61±10.07
Dissolved Oxygen	3.13±0.44
Alkalinity (mg/L)	399.57±10.64
Biological oxygen demand (mg/L)	2.47 ± 0.37
E.C (µScm−1)	290.75±15.50
Total dissolved solids (mg/L)	232.20±20.60
Turbidity	45.86±2.61

The heavy metals accumulation in water are represented in Table 2

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Table 2: Heavy metals accuulation in water of river

Metal	Main stream	Bank	WHO
Ni	0.043±0.005	0.036± 0.001	0.07
Fe	1.57±0.29	1.44± 0.24	0.10
Cd	0.41±0.049	0.39±0.019	0.03
Cu	0.151±0.022	0. 081±0.002	2
Cr	0.081±0.014	0. 075±0.0023	0.05
Pb	0.25± 0.03	0.23±0.03	0.01

Concentrations of heavy metals in sediments are presented in Table 3.

Table3: Accumulation of heavy metals in sediments of river

Metal	Main stream	Bank	WHO Standard
Ni	182.61±8.58	162.44± 10.23	30±1.0
Fe	12195±229.5	9196±266.6	7480±300
Cd	1.98±0. 06	1.76±0.005	0.6
Cu	31.51±1.22	28.48±1.50	31±1.0
Cr	39.40±1.14	36.21±2.5	67±1.0
Pb	137.85± 8.56	117.31±8.61	20

According to WHO standard the water having heavy metal pollution index greater than 1 is not suitable for drinking. The population index of nickel, iron, lead and cadmium was far greater than 1 and chromium has pollution index less than 1in sediments. Whereas, copper had greater population index in main stream while less in bank and the water was contaminated as shown in Figure 1.

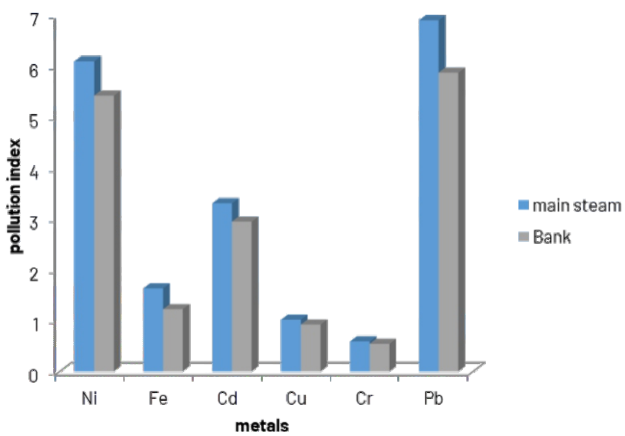

Figure 1: Comparison of pollution index bewteen sediments of main stream and bank water

Pollution Index was greater than 1 for iron, cadmium, chromium, lead and nickel while, copper have less than 1. Pollution index of water was presented in Figure 2.

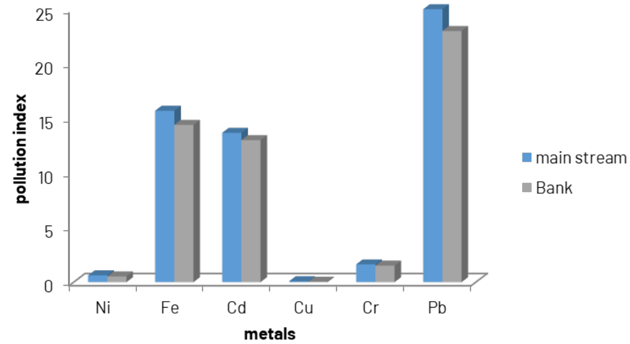

Figure 2: Comparison of pollution index bewteen water of main stream and bank water

Bioaccumulation of six heavy metals in gills, fins and muscles of *O. bimaculatus* shown in Figure 3 & 4.

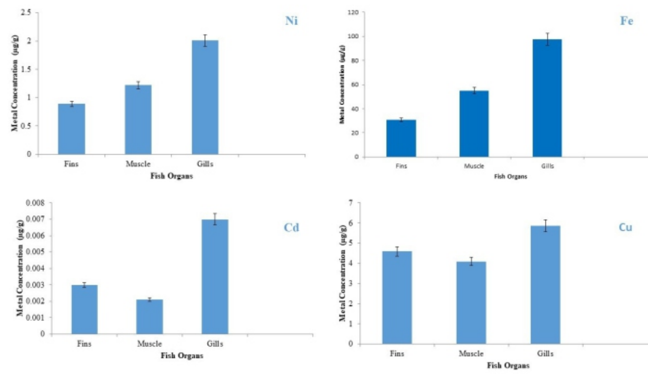

Figure:3 Accumulation of metals (Ni, Fe, Cd, Cu) in fish organs

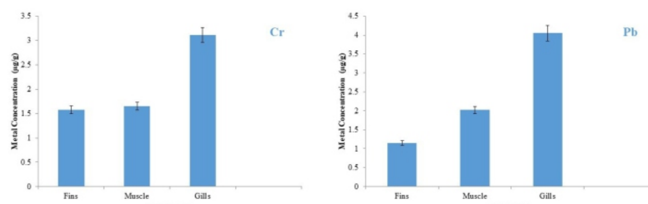

Figure 4: Accumulation of metals (Cr, Pb) in fish organs

The reference value of ACP and ALP were 35.90 and 32.90 respectively. Results of present study indicated that the value of ACP and ALP were 84.73 and 60.01 respectively which were much higher than reference value. The higher values suggest that high pollution level started the damage in fish organ have been initiated as shown in Figure 5.

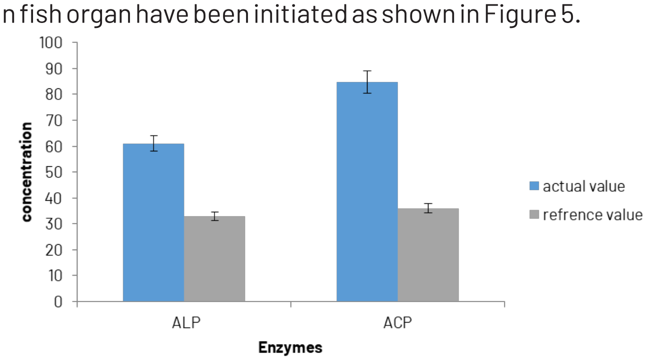

Figure 5: Level of ALP and ACP in fish blood

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DISCUSSION

Heavy metal pollution was researched in terms of buildup at Marri Pattan on the Ravi River. Metal concentrations were determined in the water, sediments, and organs of *O. bimaculatus* fish. Estimating waste levels and accumulation potential in some aquatic organisms is critical for successful waste management in river water [20]. Cadmium is mostly obtained from rechargeable nickel-cadmium batteries. Cadmium secretion in water has increased considerably due to two factors. The first is that cadmium-containing items are not recycled and are instead discharged into water. The other reason is cigarette smoking, however nonsmokers are also affected by diet [21]. My result is supported by Ahmad et al., he found the almost same results of physiochemical parameters of water [22]. Two sample locations, namely the main stream and the bank water, were chosen for this study's quality characteristics and water analysis of Ravi water. BOD, total hardness, water temperature, TDS, turbidity, carbon dioxide, water current, and phosphate were among the water quality metrics measured. Total alkalinity, chloride, conductivity, and nitrates are all factors to consider. In terms of value, all parameters were greater. Four variables, however, dissolved oxygen and pH, were mild. These results are interrelated with Sanap et al. who described the similar trend of water quality measurements in India's Gudwan river [23]. The average water temperature was below the WHO maximum range of allowed values. The total stiffness, Dissolved Oxygen and pH were linked to Jindal and Rumana [24]. Gurumayum et al. Recorded the similar values of these water parameters [25]. The measured concentrations of some heavy metals were in following order: Fe>Cu>Cr>Pb>Ni>Cd. The build-up of Ni and Cu was documented in accordance with the WHO standard (2008 and 2011). A greater nickel content could be harmful to aquatic life, lowering, for instance, skeletal calcification and gill diffusion capacity [26]. Ni may be found in electroplating, ceramics, storage batteries, steel manufacturing, and glass dyeing and colouring. The Ni content of river water is increased by all of these sources. Given that Cu has potential to kill aquatic life, various mitigating measures are necessary to reduce Cu intake into water [27]. Higher concentration of Cd were measured. Alkaline batteries, industrial electroplating, and disruption of plant metabolic systems were the main contributors to this high level [29, 30]. The concentration of Cr metal in the main stream was higher than in the bank water. Furthermore, it was found to be above WHO's allowed levels. Brraich and Jangu recorded that higher Cr levels may be attributable to Cr emission in wastewater from various tanneries or other industrial processes [27]. Jindal and Sharma reported that effluents with high Cr concentration be treated before discharge into riverine water [38]. Lead levels were found to be substantially higher than the WHO permitted limits in main stream water. Brraich and Jangu (2015) reported that It may have been caused by effluents from a number of sectors, including the production of Pb-coated items, refinement, and the paint industry [27]. With WHO (2011) reference allowable levels, almost all heavy elements, especially Cr, Pb, and Fe, were much higher than the safe water maximums. Heavy metal concentrations in water varied greatly. Heavy metal concentrations were projected to be higher prior to the monsoon season. Heavy rainfall generates a considerable amount of trash in the form of runoff water [28, 30, 31]. Tabinda et al. discovered that there was a substantial correlation between water and the buildup of Cu, Fe, Cr, and Ni in River Ravi sediments at Bloki Head Works, demonstrating that these harmful metals resuspend from the sediments into the water [32]. Same results were recorded by Ahmad et al. in *Labeo calbasu* [22]. Due to the fact that all industrial and municipal trash now directly enters river water, it is possible to deduce from a comparison of the two studies that the number of metals in the current research was higher than in the past. Fish organs and tissues acquire more metals at greater levels in freshwater ecosystems, which ultimately accumulates in the human food chain [33]. Playle concluded that fish physiological equilibrium is negatively impacted by metals in dissolved form because they are radially available to fish [34]. Fromm (1980) stated that stress that is both acute and chronic can kill fish or accumulate in many organs [35]. Evaluation of the bioaccumulation patterns of harmful metals in diverse fish species, *O. bimaculatus*, was another important goal of the study. The findings indicated that liver had the highest metal buildup. Heavy metal accumulation in fins occurred between the gills and the muscle. In muscles, the buildup was least. Fish that are carnivores accumulate metals at considerably greater rates. It could be brought on by eating contaminated seafood and decomposing organic debris. The greatest ferric concentration was measured at 97.56±4.20, copper was 5.87±0. 59 lead 4.05±0.05, chromium 3.11±0.75 and nickel 2.01±0.06. Only cadmium was a metal whose concentration in muscles was lower than the benchmark. Shivakumar et al. accompanied research on edible fish and his findings showed that muscle tissue accumulated the following amounts of heavy metals: Fe>Cu>Ni>Pb>Cd respectively [36]. In this study two enzymes; ALP and ACP also tested. These two enzymes are employed to identify the illness. When liver or tumour damage initially begins, these enzyme concentrations rise. The alkaline

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phosphatase test (ALP) is employed for the identification of liver illness and bone diseases. Liver and bone problems led to a rise in ALP levels in the blood [37]. A high level of ALP in the blood indicates the onset of one or more illness, such as a tumour. Acid phosphatase, is a lysosomal enzyme, present in the cells of the bone, spleen, kidney, liver, gut, and blood. Organ damage and tumour growth began somewhere in *O. bimaculatus*' body because the concentration of these two enzymes was higher than reference levels. Ahmad et al. also reported the higher ALP and ACP levels [22].

CONCLUSIONS

It was determined that the levels of Fe and Pb in Pakistan's riverine system were higher than those of other metals. Compared to all other organs, the concentration of all heavy metals was higher in the gills. In all fish organs, the order of metal accumulation was Fe > Pb > Cu > Ni > Cr > Cd. Additionally, flowing water had a substantially higher concentration of heavy metals than bank water.

Authors Contribution

Conceptualization: HA
Methodology: HA, SA, IA
Formal Analysis: HA
Writing-review and editing: HA, SA, IA

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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REFERENCES

[1] Verma AK. Ichthyo-faunal diversity of Alwara lake: Threats and conservation status. International Journal of Zoological Investigations. 2021 Sep.

[2] Asif M. The prevention and control the type-2 diabetes by changing lifestyle and dietary pattern. Journal of Education and Health Promotion. 2014 Feb; 3: 1. doi: 10.4103/2277-9531.127541.

[3] Banik S and Bhattacharya P. Ompok pabo (Hamilton, 1822) of Tripura, India: an endangered fish species in relation to some biological parameters. Research Journal of Biology. 2012; 2(3): 91-7.

[4] Chakrabarti NM, Chakrabarti PP, Mondal SC. Ompak bimaculatus and Ompak pabda comparative morphometric and meristic study of embryonic larval development. Fishing Chimes. 2009; 29(6): 8-9.

[5] Rauf A, Javed M, Ubaidullah M. Heavy Metal Levels in Three Major Carps (Catla Catla, Labeo Rohita and Cirrhina Mrigala) from the River Ravi, Pakistan. Pakistan Veterinary Journal. 2009 Mar; 29(1): 24-26.

[6] Chatta A, Khan M, Mirza Z, Ali A. Heavy metal (cadmium, lead, and chromium) contamination infarmed fish: a potential risk for consumers' health. Turkish Journal of Zoology. 2016 Jan; 40(2): 248-56. doi: 10.3906/zoo-1506-1.

[7] Ebong GA, Etuk HS, Johnson AS. Heavy metals accumulation by Talinum triangulare grown on waste dumpsites in Uyo metropolis, Akwa Ibom State, Nigeria. Journal of Applied Sciences. 2007 Jan; 7(10): 1404-9. doi: 10.3923/jas.2007.1404.1409.

[8] Karadede H, Oymak SA, Ünlü E. Heavy metals in mullet, Liza abu, and catfish, Silurus triostegus, from the Atatürk Dam Lake (Euphrates), Turkey. Environment International. 2004 Apr; 30(2): 183-8. doi: 10.1016/S0160-4120(03)00169-7.

[9] Khan MS, Qureshi NA, Jabeen F. Assessment of toxicity in fresh water fish Labeo rohita treated with silver nanoparticles. Applied Nanoscience. 2017 Jun; 7: 167-79. doi: 10.1007/s13204-017-0559-x.

[10] LaVaute T, Smith S, Cooperman S, Iwai K, Land W, Meyron-Holtz E, *et al*. Targeted deletion of the gene encoding iron regulatory protein-2 causes misregulation of iron metabolism and neurodegenerative disease in mice. Nature Genetics. 2001 Feb; 27(2): 209-14. doi: 10.1038/84859.

[11] Pietrangelo A. Hemochromatosis: an endocrine liver disease. Hepatology. 2007 Oct; 46(4): 1291-301. doi: 10.1002/hep.21886.

[12] Bacon BR and Schrier SL. Patient information: hemochromatosis (hereditary iron overload) (Beyond the Basics). UpToDate. 2015. 2017.

[13] Macomber L and Hausinger RP. Nickel toxicity, regulation, and resistance in bacteria. Stress and environmental regulation of gene expression and adaptation in bacteria. 2016 Aug; 20(1): 1131-44. doi: 10.1002/9781119004813.ch110.

[14] Tamers SL, Streit J, Pana-Cryan R, Ray T, Syron L, Flynn MA, *et al*. Envisioning the future of work to safeguard the safety, health, and well-being of the workforce: A perspective from the CDC's National Institute for Occupational Safety and Health. American Journal of Industrial Medicine. 2020 Dec; 63(12): 1065-84. doi: 10.1002/ajim.23183.

[15] Ala A, Walker AP, Ashkan K, Dooley JS, Schilsky ML. Wilson's disease. The Lancet. 2007 Feb; 369(9559): 397-408. doi: 10.1016/S0140-6736(07)60196-2

[16] Edwards JR and Prozialeck WC. Cadmium, diabetes and chronic kidney disease. Toxicology and applied pharmacology. 2009 Aug; 238(3): 289-93. doi: 10.1016/j.taap.2009.03.007.

DOI: https://doi.org/10.54393/pbmj.v6i08.921

[17] Leal MF, Vasconcelos MT, Sousa-Pinto I, Cabral JP. Biomonitoring with benthic macroalgae and direct assay of heavy metals in seawater of the Oporto coast (Northwest Portugal). Marine Pollution Bulletin. 1997 Dec; 34(12): 1006-15. doi: 10.1016/S0025-326X(97)00112-4.
[18] Estiarte M, Peuuelas J, Sardans J, Emmett BA, Sowerby A, Beier C, *et al*. Root-surface phosphatase activity in shrublands across a European gradient: effects of warming. Journal of Environmental Biology. 2007 Apr; 29(1): 25.
[19] Michell RH, Karnovsky MJ, Karnovsky ML. The distributions of some granule-associated enzymes in guinea-pig polymorphonuclear leucocytes. Biochemical Journal. 1970 Jan; 116(2): 207-16. doi: 10.1042/bj1160207.
[20] Biswas M. Macrozoobenthic diversity of a rural pond near Naihati, West Bengal. International Research Journal of Interdisciplinary & Multidisciplinary Studies(IRJIMS). 2015; 1: 11-4.
[21] Järup L. Hazards of heavy metal contamination. British Medical Bulletin. 2003 Dec; 68(1): 167-82. doi: 10.1093/bmb/ldg032.
[22] Ahmad S, Jafar K, Khan M, Wajid M, Waheed A, Aslam M, *et al*. Targeted accumulation of some heavy metals in Liver, Kidney, Gills and Muscles of Labeo calbasu inhibiting the freshwater riverine system. International Journal of Biosciences(IJB). 2021 Feb; 18: 11-19. doi: 10.12692/ijb/18.2.11-19.
[23] Sanap RR, Mohite AK, Pingle SD, Gunale VR. Evaluation of water qualities of Godawari River with reference to physico-chemical parameters, Dist. Nasik (MS), India. Pollution Research. 2006 Jun; 25(4): 775.
[24] Jindal R and Rumana HS. Biomonitoring of water pollution in Western Yamuna canal at Yamunanagar, Haryana. Journal of Punjab Academy of Sciences. 2000; 2(1): 177-82.
[25] Gurumayum SD, Daimari P, Goswami BS, Sarkar A, Chaudhury M. Physico-chemical qualities of water and plankton of selected rivers in Meghalaya. Journal of the Inland Fishries Society of India. 2002; 34(2): 36-42.
[26] Lynch LP. Metal accumulation in Labeo capensis Smith, 1841 and the potential use of Argulus japonicas Thiele, 1900 as a sentinel for metal accumulation from the Vaal Dam, South Africa (Doctoral dissertation, University of Johannesburg (South Africa)).
[27] Brraich OS and Jangu S. Evaluation of water quality pollution indices for heavy metal contamination monitoring in the water of Harike Wetland (Ramsar Site), India. International Journal of Scientific and Research Publications. 2015 Feb; 5(2): 1-6.
[28] Adamu CI, Nganje TN, Edet A. Heavy metal contamination and health risk assessment associated with abandoned barite mines in Cross River State, southeastern Nigeria. Environmental Nanotechnology, Monitoring & Management. 2015 May; 3: 10-21. doi: 10.1016/j.enmm.2014.11.001.
[29] Adriano DC, Adriano DC. Nickel. Springer New York; 2001; 840: 677-705. doi: 10.1007/978-0-387-21510-5_17.
[30] Islam MS, Ahmed MK, Habibullah-Al-Mamun M, Hoque MF. Preliminary assessment of heavy metal contamination in surface sediments from a river in Bangladesh. Environmental Earth Sciences. 2015 Feb; 73: 1837-48. doi: 10.1007/s12665-014-3538-5.
[31] Mohiuddin KM, Otomo K, Ogawa Y, Shikazono N. Seasonal and spatial distribution of trace elements in the water and sediments of the Tsurumi River in Japan. Environmental monitoring and assessment. 2012 Jan; 184: 265-79. doi: 10.1007/s10661-011-1966-1
[32] Tabinda AB, Bashir S, Yasar A, Hussain M. Metals concentrations in the riverine water, sediments and fishes from river Ravi at Balloki headworks. Journal of Animal and Plant Science. 2013 Jan; 23(1): 76-84.
[33] Tawari-Fufeyin P and Ekaye SA. Fish species diversity as indicator of pollution in Ikpoba river, Benin City, Nigeria. Reviews in Fish Biology and Fisheries. 2007 Feb; 17: 21-30. doi: 10.1007/s11160-006-9015-9.
[34] Playle RC. Modelling metal interactions at fish gills. Science of the Total Environment. 1998 Aug; 219(2-3): 147-63. doi: 10.1016/S0048-9697(98)00232-0.
[35] Fromm PO. A review of some physiological and toxicological responses of freshwater fish to acid stress. Environmental Biology of Fishes. 1980 Jan; 5: 79-93. doi: 10.1007/BF00000954.
[36] Shivakumar CK, Thippeswamy B, Tejaswikumar MV, Prashanthakumara SM. Bioaccumulation of heavy metals and its effect on organs of edible fishes located in Bhadra River, Karnataka. International Journal of Research in Fisheries and Aquaculture. 2014; 4(2): 90-8.
[37] Garnero P, Delmas PD. Assessment of the serum levels of bone alkaline phosphatase with a new immunoradiometric assay in patients with metabolic bone disease. The Journal of Clinical Endocrinology and Metabolism. 1993 Oct; 77(4): 1046-53. doi: 10.1210/jcem.77.4.8104954.
[38] Jindal R and Sharma C. Studies on water quality of Sutlej River around Ludhiana with reference to physicochemical parameters. Environmental Monitoring and Assessment. 2011 Mar; 174: 417-25. doi: 10.1007/s10661-010-1466-8.