An assessment of total moisture, protein, lipid, DNA, and heavy metals in *Anabas testudineus* along with the water quality at two different sites in Deepor Beel, Assam.

Dr. Evarani Kalita[1], Bratati Ray[2], Bhitumoni Choudhury[3], Salma Easmin[4] and Madhurima Devi[5]

[1]Assistant Professor, Department of Zoology, Handique Girls' College, Guwahati, India

[2,3,4,5] Undergraduate Students, Department of Zoology, Handique Girls' College, Guwahati, India

ABSTRACT

The present study focuses on the analysis of the water quality of Deepar Beel, a Ramsar site located near Guwahati city along with a comparative study of total protein, total lipid, DNA, and heavy metals in the fish species *Anabas testudineus* collected from two different sites – one is near the garbage dumping area and another one is clean area. In the study, the heavy metals were detected using the ICP- MS. Comparative quantification of total lipids was done using Folch's method, protein was determined using the Bradford method, moisture content was estimated using the oven method, and DNA was quantified using the Nanodrop. The results showed the presence of heavy metals such as Arsenic (As) and Cadmium (Cd) in both water and fish tissue. Lead (Pb) was only found in the fish muscles, whereas no evidence of Mercury (Hg) was found in the water samples and tissues collected from both sites of the Beel. The concentration of As and Cd was higher in water collected from the dumping site of the Beel in comparison with water collected from the clean site. The concentration of As, Cd, and Pb was high in the fish muscles collected near the garbage dumping site of the Beel than in the fish muscles collected from the clean site. However, the results were insignificant at 0.05 ($P>0.05$). Again, the fish muscle collected near the garbage dumping site had lower lipid, protein, and DNA levels in comparison to the fish muscle collected from the clean site, whereas the moisture content was higher in the fish muscle collected near the garbage dumping site than in the fish muscle collected from the clean site. The results were significant for lipid and protein at ($P<0.05$), whereas for moisture and DNA, the results were insignificant at 0.05 ($P>0.05$).

Keywords: *Anabas testudineus*, heavy metals, lipid, protein, moisture, DNA

INTRODUCTION

Deepor Beel is a large natural wetland of great biological and environmental importance. It is located in the Kamrup (metropolitan) district on the southern bank of the River Brahmaputra. It supports a wide variety of plants and animals. The Beel's ecosystem hosts a notable annual influx of migrating waterfowl, along with a substantial population of resident water birds. Wetlands are primarily contaminated by sewage or wastewater disposal, encroachment, and commercial and industrial activities. Wetlands face threats due to rapid population growth, urbanization, and economic expansion. The influx of wastewater from Guwahati city has led to a decline in the water quality of the Beel, threatening the aquatic life.

North-Eastern India is considered one of the global 'hot spots' of freshwater fish diversity in the world **(Kottelat & Whitten, 1996)**. The Beel supports a highly concentrated and diverse indigenous freshwater fish population, approximately 54 species belonging to 20 families. Some of the fish migrate between the wetland and the parent river Brahmaputra, and so many riverine species have also amalgamated in this wetland. These riverine species have come in along with floods **(Acharjee *et al.*, 2009)**. Freshwater fish are a vital source of protein and income for the livelihood of many indigenous peoples of Assam. The health of these people is directly linked to the well-being of the Deepor Beel wetland ecosystem, which provides essential natural resources.

The primary source of heavy metal contamination in the environment has been determined to be the exponential increase in human population, the proliferation of industrialization, expansion of agricultural practices, and a variety of geological processes **(Kumari *et al.*, 2017; Xu *et al.*, 2022)**. Heavy metals are highly toxic, persistent in the environment, and can bioaccumulate, significantly affecting water quality and safety **(Shou *et al.*, 2022)**. Maximum freshwater sources have been contaminated with heavy metals like lead (Pb), mercury (Hg), arsenic (As), cadmium (Cd), etc, which harm freshwater fish species **(Sharma *et al.*, 2024)**. The accumulation of trace heavy metals in water bodies poses significant health risks as they are absorbed by various organisms and enter the food chain **(Ahmed *et al.*, 2013; Song *et al.*, 2017; Banerjee *et al.*, 2016; Bhuyan *et al.*, 2017; Forti *et al.*, 2011; Rahman *et al.*, 2012; Yi *et al.*, 2011)**. Due to fish bio-cumulative power, some fish species accumulate higher heavy metal concentrations in their body tissues. Species in higher trophic levels accumulate the most heavy metals, posing a serious health risk to humans. Regular consumption of significant amounts of dissolved minor heavy metals can increase cancer risk. Heavy metal pollution in water systems thus poses serious environmental concerns due to its potential impacts on human health and aquatic ecosystems **(Giri & Singh, 2014; Wang *et al*., 2017; Sharma *et al*., 2024)**.

A study by **Das *et al.* (2019)** revealed that in Deepor Beel, the concentrations of all heavy metals except Mg and Cu occasionally surpassed allowable drinking water quality levels at specific sites. They observed the annual mean values of these metals in the distribution trend: Mg > Fe > Mn > Cu > Cr > Pb > Cd. The concentrations of heavy metals were found to be higher during the monsoon period at the sites near the solid waste landfill, indicating that leachability is the primary source of contamination regarding heavy metals **(Das *et al*., 2019)**. Therefore, a comprehensive study of heavy metal concentrations, their sources, and entry into the food chain, as well as their effects on water quality, is essential to mitigate potential future contamination and preserve natural water resources.

The present study focuses on a comparative analysis of heavy metals between the two different sites of the Deepor Beel, i.e, near the garbage dumping site and the clean site. Besides, we also performed some biochemical experiments on the fish species *A. testudineus* collected from those two sites. The fish species was selected due to its high consumption by local communities. In addition to biochemical parameters, we also studied heavy metal concentrations in fish tissues collected from those two sites.

MATERIALS AND METHODS

Study area

Deepor Beel is located between the latitudes of 26°05′26″N to 26°09′26″N and longitudes of 90°36′39″E to 91°41′25″E, at an altitude of 165-186 feet above Mean Sea Level. The Beel holds significant biological and environmental importance. It is a permanent freshwater lake located in a former channel of the Brahmaputra River, to the south of the main river. It is the only major stormwater storage basin for the city of Guwahati in the Kamrup Metropolitan district of Assam, India. It covers an approximate area of about 4.14 square kilometers. In 1989, the area was declared a wildlife sanctuary by the Government of Assam. It is listed as a wetland under the Ramsar Convention, which designated the lake as a Ramsar Site in November 2002.

Collection of Surface water

The surface water collection was collected during the month of May 2023, both from the clean and garbage dumping areas of the Beel. The samples were collected and analyzed as per the procedures specified in "Standard Methods for the Examination of Water and Wastewater" published by **APHA (2005)**. Samples for chemical analysis were collected in polyethylene carboys. Samples collected for heavy metals (As, Hg, Pb, and Cd) analysis were acidified with HNO_3(1ml HNO_3/100 ml).

Collection of fish samples

Anabas testudineus were selected to carry out the experiment. The fish samples were collected separately from both the clean and garbage-dumping areas of the Beel. The tissues (muscles) were separated, cleaned, and preserved at -20°C for further testing.

Heavy Metal estimation

The presence of heavy metals (As, Hg, Pb and Cd) in the water and fish tissue samples was performed at the NABL-accredited laboratory of Central Ground Water Board (CGWB), NER, Guwahati, Assam, using the ICP- MS. Before the analysis, the tissue samples were subjected to wet digestion.

Biochemical estimation

The Oven method was used to estimate the moisture content of the collected fish samples **(AOAC, 2005)**. Total lipid content was estimated using the Folch method **(Folch *et al*., 1957)**. Tissue protein was estimated following the Bradford method **(Bradford, 1976)**. The DNA concentration of the samples was estimated using Nanodrop **(García-Alegría *et al*., 2020)**. The data were statistically analyzed using the mean ± S.D. (Standard Deviation). A one-way ANOVA test was conducted to identify significant differences between means using SPSS.

RESULTS

Heavy metal content of the water and the body muscles of *A. testudineus*

In the present study, we recorded higher concentrations of As and Cd in the water samples collected near the garbage dumping site compared to those collected from the clean site of the Beel. (Table 1). We also observed a higher concentration of As, Pb, and Cd in the muscles of fish collected from the dumping site compared to those collected from the clean site of the Beel (Table 2). However, the results were insignificant at 0.05 ($P>0.05$). The recorded concentrations of the heavy metals in the present experiment were below the permissible limit.

The permissible limits (ppm) for the heavy metals (As, Hg, Pb, and Cd) in food, water, and soil according to international standards (Codex Alimentarius, 2015) are:

Matrices	Arsenic (As)	Cadmium (Cd)	Mercury (Hg)	Lead (Pb)
Food	0.1-0.2	0.05-2	0.5-1	0.01-3
Water	0.01-0.1	0.003-0.01	NA	0.01-5
Soil	NA	0.9-3	0.03-2	30-50

NA: No conclusion available

Table 1: Heavy Metal concentration in water samples collected near the Garbage dumping site and the Clean site of the Beel.

Water Sample Collected Site	As (ppb)	Hg (ppb)	Pb (ppb)	Cd (ppb)
Near garbage Dumping	0.04± 0.05	BDL	BDL	0.13± 0.008
Clean	0.02 ± 0.09	BDL	BDL	0.10± 0.003

Each value is the mean ±SD of six observations. BDL, below detectable limit.

Table 2: Heavy Metal concentrations in the fish muscles collected near the Garbage dumping site of the Beel.

Fish collected Site	As (ppb)	Hg (ppb)	Pb (ppb)	Cd (ppb)
Near garbage Dumping	0.47±0.01	BDL	0.06±0.07	1.74±0.08
Clean	0.37±0.03	BDL	0.01±0.02	1.61±0.005

Each value is the mean ±SD of six observations. BDL, below detectable limit.

Moisture, lipid, protein, and DNA contents of the body muscles of *A. testudineus*

In the present study, we recorded that the lipid and protein content in the fish muscles collected near the garbage dumping site of the Beel is significantly (P>0.05) lower than that in the fish muscles collected from the clean site (Fig. 1). The DNA content (Fig. 2) was also lower in the fish muscles collected near the garbage dumping site of the Beel than that in the fish muscles collected from the clean site, but it was insignificant at 0.05 (P>0.05). In contrast, the moisture (Fig. 1) content in fish muscles collected near the garbage dumping site of the Beel was higher than that of fish muscles collected from the clean site. However, the results are insignificant at 0.05 (P>0.05).

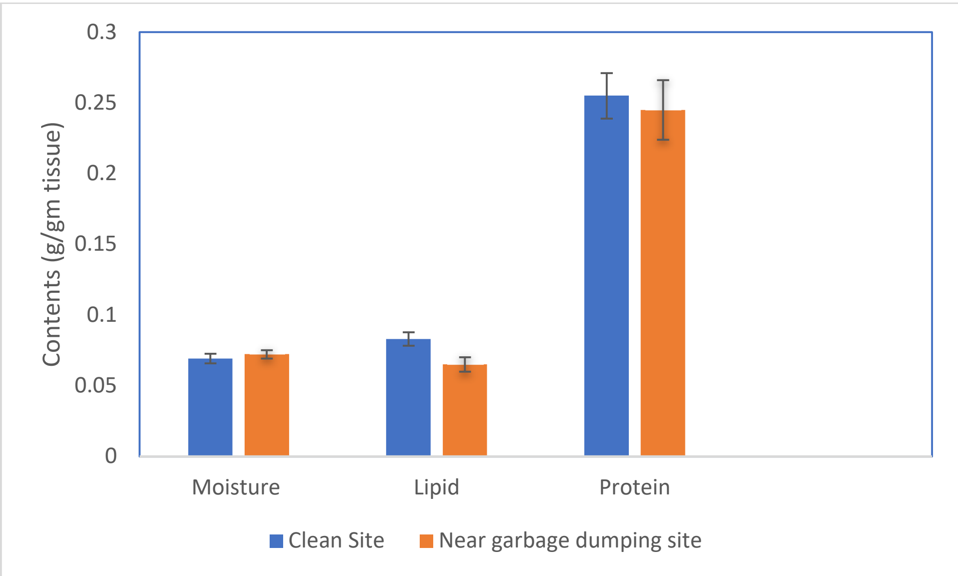

Fig.1. Comparative study of total moisture, lipid, and protein in the muscles of *A.testudineus* in two different sites of the Beel. Each value is the mean ±SD of six observations.

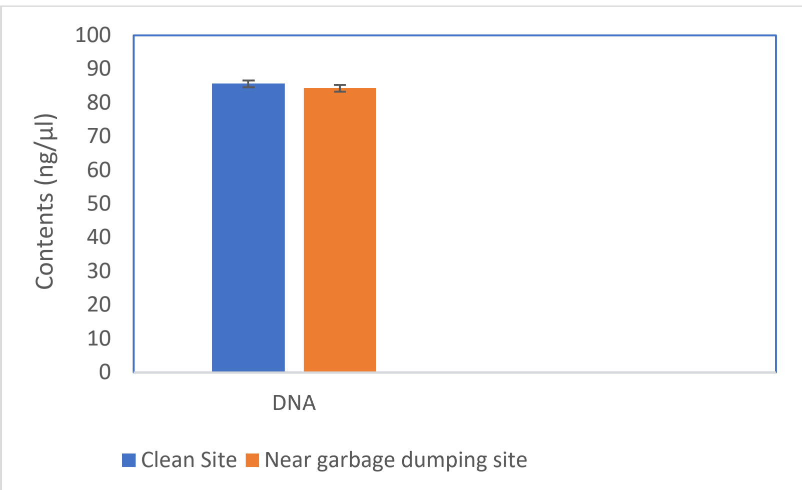

Fig.1. Comparative study of DNA content in the muscles of *A. testudineus* in two different sites of the Beel. Each value is the mean ±SD of six observations.

DISCUSSION

In the present experiment, an analysis of water collected from two different sites of Deepor Beel revealed that the concentrations of As and Cd are higher in the water samples collected near the garbage dumping site than in the samples collected from the clean site of the Beel. It may be due to the leakage of heavy metals from the Garbage towards the Beel. This finding was consistent with the observations of **Gohain & Bordoloi (2017),** who found heavy metals Mn, Zn, Cr, Ni, Cu, and Cd in the surface water of the Beel in decreasing concentrations with increasing distance from the source of the municipal solid waste dumping site. In the present study, we found Pb in the muscles of the fish collected from both sites, although in the water samples, Pb was below the detectable limit. It may be due to the accumulation of metals in their muscles. **Jamil et al (2023)** reported that fish accumulate metals through several important body tissues (gills, liver, kidney, skin, muscle, etc.). The accumulation of these metals has destructive effects on human health as well as the organisms themselves **(Farombi et al, 2007)**. Heavy metal contamination negatively affects the growth and reproductive activity of fish. However, the severity of metal toxicity (carcinogenic, teratogenic, and mutagenic) varies significantly with the fish species, the concentration of the metals, and the duration of exposure (**Ngo et al, 2011).** Although various metals are essential for living organisms **(Rohani et al, 2022)**, most can be very dangerous, even in small amounts **(Lakherwal, 2019)**. Moreover, some metals, namely As, Cd, Cu, Cr, Pb, Hg, Ni, Se, Zn, etc., are not only highly toxic but also cause carcinogenicity and mutagenicity **(Korte et al, 2020).**

In the present study, we recorded significantly lower lipid and protein content in the fish muscles collected from the garbage dumping site of the Beel compared to the fish muscles collected from the clean site of the Beel. The present results align with the findings of **Tulasi et al (1992)**, who reported that exposure of fish *A. testudineus* to a sublethal concentration of lead nitrate for 30 days during the preparatory phase of its annual reproductive cycle reduced the total lipids. In a study, **Akter et al. (2009)** reported a decline in protein content in *A. testudineus* with increasing concentrations of As and Hg, which is consistent with our current findings. The present results also align with the findings of **Tulasi et al (1992)**, who reported that exposure of fish *Anabas testudineus* to a sublethal concentration of lead nitrate for 30 days during the preparatory phase of its annual reproductive cycle reduced the total lipids.

The DNA content was also lower in the fish muscles collected from the garbage dumping site of the Beel, but was insignificant at 0.05 ($P>0.05$). Heavy metals can lower the capacity of cells to repair DNA (**Jadoon & Malik, 2017**). The cell consists of proteins and enzymes, and their function is to maintain the DNA. However, many external factors and endogenous processes continuously damage the DNA. Heavy metals enter the body and damage DNA due to the formation of reactive oxygen species. DNA damage can increase the risk of cancer.

In contrast to lipid, protein, and DNA content, the moisture content in fish muscles collected near the garbage dumping site of the Beel was higher than that in fish muscles collected from the clean site. However, the results were insignificant at 0.05 ($P>0.05$). In a study, **Bezbaruah and Deka (2021)** mentioned that the high moisture content in fish increases the rate of deterioration when stored for extended periods. This is due to the heightened activity of microorganisms in high moisture environments.

Heavy metal poisoning in fish varies significantly, leading to physiological, biochemical, cellular, and molecular changes in their bodies (**Phoonaploy et al., 2019; Kamaraju and Ramasamy, 2018**). These metals can bind with biological particles that contain nitrogen, sulfur, and oxygen, thereby altering the structure and function of proteins, enzymes, and hormones, ultimately damaging various organs of fish (**Banday et al, 2019).**

CONCLUSION

Heavy metal contamination in aquatic ecosystems poses significant environmental challenges due to its harmful effects on ecosystems and human health. Since heavy metals are non-biodegradable, they can cause toxicity in faunal communities. Toxicity in aquatic organisms varies based on their traits and the characteristics of their water environment. Fish are one of the most widely distributed species in aquatic environments, rendering them more vulnerable to heavy metals. Fish can easily metabolize, detoxify, and accumulate heavy metals in their bodies, making them useful bioindicators for environmental monitoring. Aside from their toxic effects, the presence of heavy metals increases the generation of reactive oxygen species (ROS) in both aquatic life and humans, resulting in oxidative stress. This condition greatly increases the risk of several health issues, including cancer, kidney damage, liver disease, and skin problems. Besides water, living organisms absorb toxic metals from soil and air through external surface contact, digestion, and the inhalation of airborne metal particles. The majority of heavy metals occur naturally, but some originate from human activities, primarily commercial and industrial sources. Hence, it is essential to raise scientific awareness among individuals to develop and implement effective strategies for minimizing this environmental issue.

ACKNOWLEDGEMENTS

The authors acknowledge the financial assistance received from the Research and Development Cell, Handique Girls' College, Guwahati. They are also thankful to the NABL-accredited laboratory of Central Ground Water Board (CGWB), NER, Guwahati, Assam, for ICP- MS; and the laboratory facilities of Advanced Level Institutional Biotech Hub and Department of Zoology, Handique Girls' College, Guwahati.

COMPETING INTERESTS

There is no conflict of interest, as we do not intend to use them for litigation purposes but rather to advance knowledge.

AUTHORS CONTRIBUTIONS

The authors confirm their contribution to the paper as follows:

- EK: Experimental design, interpretation of data, and manuscript writing
- BR: Statistical analysis and ICP- MS experiment
- BC: Water Sample collection and lipid estimation
- SE: Fish Sample collection and DNA estimation
- MD: Protein and moisture estimation

REFERENCES

Acharjee B., Dutta A., Sharma D. K, Das P. & Behera B. K. (2009). Ecological Status of Deepor Beel, Assam With special reference to its fish and fisheries. In book: Wetlands of North East India: Ecology, Aquatic Bioresources and Conservation (pp.41-54) Publisher: Akansha Publishing House, New Delhi, Editors: L. Kosygin

Ahmed, M.K., Habibullah-Al-Mamun, M., Parvin, E., Akter, M.S., Khan, M.S. (2013). Arsenic induced toxicity and histopathological changes in gill and liver tissue of freshwater fish, tilapia (*Oreochromis mossambicus*). *Exp Toxicol Pathol*. 65(6):903–9.

Akter, M.S., Ahmed, K., Akhand, A.A., Ahsan, N., Islam, M. and Khan, S.(2009). Arsenic and Mercury Induce Death of *Anabas testudineus* (Bloch) Involving Fragmentation of Chromosomal DNA. *Terrestrial And Aquatic Environmental Toxicology*, 3(1), 42-47, Global Science Books.

AOAC (2005). Official Methods of Analysis. 18th edn. Association of Official Analytical Chemists; Arlington, VA, USA: 2005.

Banday, U.Z., Swaleh, S.B., Usmani, N. (2019). Insights into the heavy metal-induced immunotoxic and genotoxic alterations as health indicators of Clarias gariepinus inhabiting a rivulet. Ecotoxicol. Environ. Saf. 183, 109584.

Banerjee, S., Kumar, A., Maiti, S. K., and Chowdhury, A. (2016). Seasonal variation in heavy metal contaminations in water and sediments of Jamshedpur stretch of Subarnarekha river, India. Environmental Earth Sciences, 75(3). https://doi.org/10.1007/s12665-015-4990-6

Bezbaruah, & Deka, D.D. (2021). Variation of moisture and protein content in the muscle of three catfishes: A comparative study. *Int J Fish Aquat Stud,* 9(1):223-226. DOI: https://doi.org/10.22271/fish.2021.v9.i1c.2406

Bhuyan, M. S., Bakar, M. A., Akhtar, A., Hossain, M. B., Ali, M. M., Islam, M. S. (2017). Heavy metal contamination in surface water and sediment of the Meghna River, Bangladesh. Environmental Nanotechnology, Monitoring & Management, 8, 273–279. https://doi.org/10.1016/j.enmm.2017.10.003

Bradford, M. (1976). "A Rapid and Sensitive Method for the Quantification of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding” Analytical Biochemistry, 72(1-2):248-254, 254, doi:10.1006/abio.1976.9999. PMID 942051

Codex Alimentarius (2015). Codex general standard for contaminants and toxins in food and feed - CODEX STAN 193-1995. Joint FAO/WHO; p. 59. Retrieved from 10.13140/RG.2.1.4910.2560

Dash, S., Borah, S.S. and Kalamdhad, A. (2019). A modified indexing approach for assessment of heavy metal contamination in Deepor Beel, India, Ecological Indicators, Volume 106, 105444, ISSN 1470-160X, https://doi.org/10.1016/j.ecolind.2019.105444.

Eaton, A.D., Franson, M.A.H., Clesceri, L.S., Rice, E.W. and Greenberg, A.E. (2005). Standard methods for the examination of water & wastewater, 21st ed./Centennial ed. Published by American Public Health Association (APHA), Washington, DC, ©2005

Farombi, E.O., Adelowo, O.A. & Ajimoko Y.R.(2007). Biomarkers of Oxidative Stress and Heavy Metal Levels as Indicators of Environmental Pollution in African Cat Fish (*Clarias gariepinus*) from Nigeria Ogun River. Int. *J. Environ. Res. Public Health*. 4:158–165. doi: 10.3390/ijerph2007040011.

Folch, J., Lees, M. and Stanley, G.H.S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Bio. Chemis*. 226(1):497-509. PMID: 13428781

Forti, J. C., Lima, P. G., Reis, A. R., Santos, F. A., & Braga Junior, S. S. (2019). Analysis of heavy metals and aromatic compounds in soil layers of a sanitary landfill. Environmental Quality Control. https://doi.org/10.1002/tqem.21607

García-Alegría, A.M., Anduro-Corona, I., Pérez-Martínez, C.J., Guadalupe Corella-Madueño, M.A., Rascón-Durán, M.L. and Astiazaran-Garcia, H. (2020). Quantification of DNA through the NanoDrop Spectrophotometer: Methodological Validation Using Standard Reference Material and Sprague Dawley Rat and Human DNA. *Int J Anal Chem.* 8896738. doi: 10.1155/2020/8896738. PMID: 33312204; PMCID: PMC7719535.

Giri, S., and Singh, A. K. (2014). Assessment of surface water quality using Heavy Metal Pollution Index in Subarnarekha River, India. Water Quality, Exposure and Health, 5, 173–182. https://doi.org/10.1007/s12403-013-0106-2

Gohain, S.B. & Bordoloi, S. (2017). Higher concentration of heavy metals in surface water and fish near a municipal solid waste dump in Guwahati, Assam, India. *Current Science*, Vol. 113, No. 9

Jadoon, S and Malik, A. (2017). DNA Damage by Heavy Metals in Animals and Human Beings: An Overview. Biochemistry & Pharmacology, 6(3), ISSN: 2167-0501

Jamil, E.F., Rohani, M.F., Sumaiya, N., Tuj, J.M.F., Akter, Y., Shahjahan, M., Abdul, K. Z., Tahiluddin, A.B. & Goh, K.W. (2023). Bioaccumulation and Bioremediation of Heavy Metals in Fishes-A Review. *Toxics.* 11(6):510. doi: 10.3390/toxics11060510. PMID: 37368610; PMCID: PMC10302055.

Kamaraju, S and Ramasamy, K. (2018). Effect of Heavy Metal, Cadmium Chloride on Protein and Amino Acid Content Changes in Freshwater Exotic Fish, Hypopthalmichthys Molitrix, Indo Am. J. P. Sci, 05(02).

Kortei, N.K., Heymann, M.E., Essuman, E.K., Kpodo, F.M., Akonor, P.T., Lokpo, S.Y., Boadi, N.O., Ayim-Akonor, M. & Tettey C (2020). Health Risk Assessment and Levels of Toxic Metals in Fishes (Oreochromis niloticus and Clarias anguillaris) from Ankobrah and Pra Basins: Impact of Illegal Mining Activities on Food Safety. *Toxicol. Rep.*

7:360–369. doi: 10.1016/j.toxrep.2020.02.011.

Kottelat, M. and Whitten, T. (1996). Freshwater Biodiversity in Asia with special reference to Fish: World Bank Tech. Paper No. 343. The World Bank, Washington, DC, 17-22 p.

Kumari B., Kumar, V., Sinha, A.K., Ahsan, J., Ghosh, A.K., Wang, H., et al. (2017). Toxicology of arsenic in fish and aquatic systems. *Environ Chem Lett.* 15:43–64.

Lakherwal, D. (2019). Adsorption of Heavy Metals-a Review. *Mater. Today Proc.* 18:4745–4750. doi: 10.1016/j.matpr.2019.07.462.

Ngo, H.T.T., Gerstmann, S. & Frank, H. (2011). Subchronic Effects of Environment-like Cadmium Levels on the Bivalve Anodonta anatina (Linnaeus 1758): III. Effects on Carbonic Anhydrase Activity in Relation to Calcium Metabolism. *Toxicol. Environ. Chem.* 93:1815–1825. doi: 10.1080/02772240802503619.

Phoonaploy U, Tengjaroenkul B, Neeratanaphan L. (2019). Effects of electronic waste on cytogenetic and physiological changes in snakehead fish (Channa striata). Environmental Monitoring and Assessment. 191(6):1–11. https://doi. org/10.1007/s10661-019-7509-x

Rahman, M. S., Molla, A. H., Saha, N., and Rahman, A. (2012). Study on heavy metals levels and its risk assessment in some edible fish from Bangshi River, Savar, Dhaka, Bangladesh. Food Chemistry, 134(4), 1847–1854. https://doi.org/10.1016/j.foodchem.2012.03.099

Rohani, M.F., Bristy, A.A., Hasan, J., Hossain, M.K. & Shahjahan, M. (2022). Dietary Zinc in Association with Vitamin E Promotes Growth Performance of Nile Tilapia. *Biol. Trace Elem. Res.* 200:4150–4159. doi: 10.1007/s12011-021-03001-9.

Kamaraju, S. and Ramasamy, K. (2018). Effect of Heavy Metal, Cadmium Chloride on Protein and Amino Acid Content Changes in Freshwater Exotic Fish, Hypopthalmichthys Molitrix, Indo Am. J. P. Sci, 05(02).

Sharma, A.K., Sharma, M., Sharma, S., Malik, D.S., Sharma, M., Sharma, M. and Sharma, A.K. (2024). A systematic review on assessment of heavy metals toxicity in freshwater fish species: Current scenario and remedial approaches, Journal of Geochemical Exploration, Vol 262, ISSN 0375-6742, https://doi.org/10.1016/j.gexplo.2024.107472.

Shou, Y., Zhao, J., Zhu, Y., Qiao, J., Shen, Z., Zhang, W., Han, N. and Núñez-Delgado, A. (2022). Heavy metals pollution characteristics and risk assessment in sediments and waters: The case of Tianjin, China. *Environmental Research*, *212*, p.113162.

Song Y, Wang Y, Mao W, Sui H, Yong L, Yang D, Jiang D, Zhang L, Gong Y. (2017). Dietary cadmium exposure assessment among the Chinese population. PLoS One. 2017 May 18;12(5):e0177978. doi: 10.1371/journal.pone.0177978. PMID: 28542445; PMCID: PMC5436861.

Tulasi, S.J., Reddy, P.V. and Rao, J.V. (1992). Accumulation of lead and effects on total lipids derivatives in the freshwater fish *Anabas testudineus*. *Ecotoxicol. Environ. Safe,* 23: 33- 38.

Wang, Z., Luo, P., Zha, X., Xu, C., Kang, S., Zhou, M., Nover, D., and Wang, Y. (2022). Overview assessment of risk evaluation and treatment technologies for heavy metal pollution of water and soil. Journal of Cleaner Production, 379(Part 2), 134043. https://doi.org/10.1016/j.jclepro.2022.134043

Xu F. Wang Y. Chen X. Liang L. Zhang Y. Zhang F. Zhang T. (2022). Assessing the environmental risk and mobility of cobalt in sediment near nonferrous metal mines with risk assessment indexes and the diffusive gradients in thin films (DGT) technique, Environ Res,212(Pt C):113456. doi: 10.1016/j.envres.2022.113456.

Yi, Y., Yang, Z., and Zhang, S. (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), 2575–2585. https://doi.org/10.1016/j.envpol.2011.06.011

****