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Determination of LC_{50} of Copper Sulphate for a freshwater Carp, *Labeo rohita*

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Abstract-Aquatic pollution is considered as a major issue due to its detrimental effects on both aquatic and terrestrial organisms including human beings. Several industries are directly discharging the pollutants such as heavy metals into the water bodies without any prior treatments especially in developing countries. Heavy metal such as copper cause toxicity and adverse impact on fish health. The aim of present study was to determine of behavior and 96 hour lethal concentration (LC_{50}) value of copper sulphate for the freshwater fish, *Labeo rohita* (rohu). The fingerlings of *L. ohita* with average total length ± standard deviation (SD), 13.843 ± 1.162 cm; average weight ± SD, 24.38 ± 4.516 g were taken in six groups having 10 individual in each. Fish specimens were exposed to copper sulphate as 0, 1, 2, 3, 4, 5 and 6 mg/L for 96 hours after acclimatization. Mortality and behavior of fish was recorded. The irregularity in swimming, loss of balance and excessive body secretions was seen in copper sulphate exposed fish individuals. The lethal concentration value of copper sulphate for the fish, *L. rohita* was estimated as 1.60 mg/l. The current results provide acceptance limit of maximum quantity of copper for *L. rohita*. The pretreatment of industrial effluents was recommended before discharge.

Index Terms**:** Toxicity, copper sulphate, Heavy metal, Fish, *Labeo rohita*,

I. INTRODUCTION

Heavy metals are persistent and continuous environmental contaminants that can produce a variety of dysfunctions in target tissues of exposed animals [1]. Heavy metals are well-known for their carcinogenic and other toxicological consequences [2]. The phrase "heavy metal" refers to metalloids that can cause toxicity in human beings, animals, and the entire environment [3]. The cadmium, copper, lead, nickel, chromium, zinc, arsenic and mercury are primary heavy metals. Some critical metals (iron, zinc, copper, and so on) are required in biological systems, but their excessive usage is harmful [4]. While lead, mercury, cadmium, and other non-essential metals even at trace levels are hazardous to biological systems [5]. In short, most of the heavy metals are hazardous to life and environment. Metallic components enter into the body of animal or human and are accumulate in different body organs mostly in skin, hair, brain, lungs, adrenal gland, liver and kidneys. The interactions of heavy metals with enzymes, hormones and proteins impair the regular activities of enzymes and hormones in a variety of ways. They can create stable compounds with proteins, hormones and enzymes [6]. Environmental contamination has been one of the most catastrophic crises confronting human life in recent decades. This involves contamination of the land, air, and water. However, due of the inter play of numerous contaminants that occur in the aquatic environment [7]. The assessment of pollution in aquatic ecosystem is not easy as compared to the land and air [8]. Pollution caused by organic and inorganic pollutants and heavy metals has harmed both farmed and wild fish. Fish are rich source of proteins and one of major component of food web; humans depend upon fish directly and indirectly for protein [9]. The accumulated metals in tissues of body organs made them an ecological marker for pollution [10], [11], [12], [13].

Fish is a good source of protein and utilized world widely as a healthy food [14]. The forming of common carps are promoted in all over Asia including Pakistan and most areas of Europe [15], due to their economic importance. The industrial or agricultural effluents have sever heavy metals and increase the level of pollutants in rivers, lakes and oceans which have toxic effect on fish health [16]. The development in the field of industrial and agriculture discharged the industrial effluent without any treatment in the water bodies is a major cause of aquatic pollution in Pakistan that are harmful for fish health [7]. Heavy metals are discharged into the water system through anthropogenic behavior of human that beyond the permissible limits [17]. The insistence of heavy metals in water bodies can have long-term effects on biogeochemical cycling, which can have a major influence on fish development [18]. Organic pollutants induce bacterial and viral growth and have an impact on the fish population [16]. Heavy metal toxicity in fish is multifaceted, manifesting as multiple alterations in the physiological and chemical processes of their bodily systems [19]. Fish may absorb substantial levels of heavy metals from contaminated water [20] and are thought to be a good indication of pollution caused by heavy metals in aquatic ecosystems [21], [22], [23].

Copper, considered as essential trace metal for life and it is found in natural waters, sediments, air and soil [24], [25]. However, it would become toxic to organisms as the concentration of Cu crossed the permissible limit in an aquatic ecosystem, it increased free radicals production in the body [26], teratogenicity [27] and chromosomal aberrations [28]. Moreover, copper become a stressor agent at higher concentration that can impair the biological functions and cause some pathological changes in tissues [29]. Histopathological alterations in animal tissue, particularly fish tissue, are strong predictors of past exposure to aquatic environmental stresses.

Furthermore, because it permits the recognition of particular organelles, cells and organs infected in vivo, histopathological study showed the outcome of detrimental biochemical and physiological alterations in an exposed individual. Copper' has a fatal impact either directly or indirectly on aquatic species. The gills of a fish respond directly to the water pollution and to be impacted by copper firstly, because copper has the potential to control the circulatory and neural systems of fish, it may have an effect on them once it accumulates in the gills. The accumulated copper may have an effect on the circulatory and nervous system of the fish due to the ability to control the in and out transportation of salt (NaCl). In addition, copper can affect the reproduction in many fish species by decrease the production of sperm and egg e.g. fathead minnows [30]. The metabolism of glucose and cellular composition of fish was affected by copper [29].

The different level the bio-accumulation of copper different body organs showed diverse histopathological alterations in fish. The gills of a fish are the first organ that accumulates heavy metals at a higher level as compared to the concentration deemed toxic through assimilation along the gill surface and gut tract wall [31]. Heavy metals accumulated in the gills were affected the respiration and osmoregulation processes were disturbed due to the accumulation of metals in gill tissue that cause damage to gill cells [32], [33]. Copper also has histopathological impact on liver tissue; the liver is one of the most vital linked with the detoxification and biotransformation process [34].

The lethal concentration of metals may fluctuate from fish species to species and its toxic impact also varied from organ to organ [35]. The long term consumption or use of contaminated fish with toxic metals shows several adverse effects [36], [37], [38]. As *Labeo rohita* have higher demand of consumers in Punjab, Pakistan, the present study was aimed to find out the tolerance limits of *Labeo rohita* against the toxicity of copper to propose measures about their sustainable protection.

II. MATERIALS AND METHODS

A. Fish samples and acclimation conditions

The healthy individuals of *Labeo rohita* with average total length ± standard deviation (SD), 13.843 ± 1.162 cm; average wet body weight ± (SD), 24.38 ± 4.516 g were purchased from local Fish Seed Hatchery, Lahore and transported to the Fish Biology laboratory, Institute of Zoology, University of the Punjab Lahore.

Prior to the experimentation, the specimens were acclimated in a laboratory conditions after stocking in glass aquaria containing tap water for 14 days to minimize stress in fish specimen. Water quality parameters *viz.*, temperature 20±1 ◦C, dissolved oxygen 7.3 mg/l and pH 7.8 were recorded .using APHA [39]. Fish were fed twice a day for the period of acclimatization under constant photoperiod of 12D:12L in the laboratory.

B. Test chemical and stock solution

The copper sulphate $CuSO_4.5H_2O$ (Sigma Aldrich) was used in this study to determine the lethal concentration (LC_{50}) of copper. Stock solution of $CuSO_4.5H_2O$ was prepared in deionized water and maintained the essential concentration in experimental aquaria.

C. Experimental design

The fish specimens were grouped in to six groups (I, II, III, IV, V and VI) having 10 individuals in each group. Groups II to V I were exposed to $CuSO_4.5H_2O$, as 1 mgL^{-1}, 2 mgL^{-1}, 3 mgL^{-1}, 4 $mgL^{-1}$and 5 mgL^{-1}, respectively for 96 hours, while group I was free of copper as a control. No food was provided during the experiment under constant temperature which was maintained at 30 ºC. The water of each group was changed by maintaining the desired concentration of copper salt. The physico-chemical parameters of water were monitored throughout the experimental period after 12 h intervals following APHA [39].

D. Determination of 96 h-LC_{50} of Copper Sulphate

The fish mortality was noted at 12, 24, 48, 72 and 96 hours and the LC_{50} was estimated employing Probit analysis [40]. The fish behavior also monitored at 12, 24, 48, 72 and 96 [41].

III. RESULTS AND DISCUSSION

In current study, fish exposed to copper sulphate for 96 hours demonstrated aberrant behavior such as irregular swimming and loss of balance during the first hour. Fish congregated at the surface and struggled to breathe. They were drowsy, and mucus material was released throughout the body. The copper sulphate exposed fish specimens appeared on water surface more frequently than the control fish. The control group showed no discernible behavioral changes (Table 1). The fishes' behavioral response to copper sulphate revealed that they were worried and restless. Copper sensitivity is determined by the movement of fishes in water body, homeostatic regulation of its intake, excretion and storage [42], the regulation of membrane permeability, and the ratio of permeable membrane to body size [43]. Boisen et al. [44] reported that marine fish were usually showed less sensitivity to water borne copper exposure than fresh water fish. Similar changes in different fish species were observed with exposure to other metals. Yakoob et al. [45] reported the behavioral changes in Chromium exposed *Catla catla*, *Labeo rohita* and *Cirhinus mrigala*. The observations of Mishra and Mohanty [46] also support our results as they reported the changes in behavior of fish exposed to hexavalent chromium.

The mortality with response to toxic impact of copper, expressed in acute toxicity bioassays of copper sulphate for 96 hours of *L. rohita* was noted. In the group II (2 mg/L) during the 96 hours acute exposure, 7 fish died. While in all other groups, I, III, IV, V and VI groups (1, 3, 4 and 5 mg/L respectively), all fish had died. There is no mortality were observed in the control group (Table 2). The might be associated with damage of respiratory epithelium. It is main cause of mortality as oxygen culminates in the development of a mucus layer over the gills of fish. Boeck and Opdecam [47] noted decrease in oxygen contents of the water during the acute exposure of waterborne Cu for *Cyprinus carpio*. The limits of fish to tolerate the specific heavy metals directly interlinked with various feature i.e. pH, alkalinity, temperature and total hardness as a species specific ability of survival under various concentration of metallic toxicity [48].

Table 1: Impact of the Copper sulphate on the behavior of *Labeo rohita*

Parameters	Groups					
	I	II	III	IV	V	VI
loss of balance	–	+	+	++	+++	+++
Hyperactivity	–	+	+	++	+++	+++
rate of swimming	+	+	+	++	+++	+++
Convulsion	–	+	+	++	+++	+++
Secretions	–	+	+	++	+++	+++
Opercular activity	+	++	++	+++	+++	+++

(+) mild, (++) moderate, (+++) strong, (−) None.

Table 2: Effects of different concentration of Cuso4.5H2O on Mortality rate at different time period (12 – 96h).

Groups	Copper sulphate Conc. (Mg/l)	No. of fish exposed to Cu	Mortality at					Total dead fish	Total living fish	Mortality (%)	Probit kill
			12 hr	24 hr	48 hr	72 hr	96 hr				
I	0	10	0	0	0	0	0	0	10	0	0
II	1	10	0	0	0	8	10	10	0	100	7.37
III	2	10	3	5	5	7	7	7	3	70	5.52
IV	3	10	5	9	10	10	10	10	0	100	7.37
V	4	10	2	10	10	10	10	10	0	100	7.37
VI	5	10	6	9	10	10	10	10	0	100	7.37

During this study, the 96-hours LC_{50} of copper sulphate for the *L. rohita* was recorded to be 1.60 mg/L (Fig. 1). The regression line expressed the relation between the Probit kill of fish *L. rohita* (mortality percentage) and log concentration of copper sulphate.(Fig. 1). Similarly, the median lethal concentration (LC_{50}/96hrs) by using Finney's Probit analysis for *Labeo rohita* (embryonic and larval, Swim-up fry, advance fry, and fingerling) was estimated and reported 0.37, 0.75, 1.07, and 1.34 ppm respectively. These findings support the present result. The sensitivity of fish was varied for species and metals and its concentration. Earlier researchers reported as LC_{50} value of copper sulphate for *Labeo rohita* were 0.56 mg/l and 3.15 mg/l [50], [51], 96 hours LC_{50} of Lead Nitrate 378 mg/l was found for *Clarias batrachus* and 2.624 mg/l was estimated for *Cyprinus carpio* [52], [53], [54]. There are variations in the value of LC_{50} of same heavy metal was observed. Das and Banerjee [55] described that heavy metal have showed heavy toxic effects to some fish less toxic for another species and similarly the fish species showed the high sensitivity towards toxicity of one metal and less sensitivity against the toxicity of equally toxic heavy metal at the same concentration [56].

Fish are comparatively very responsive to the alterations in their adjoining surroundings. Fish health is a good indicator for a specific aquatic ecosystem to estimate the pollution. Early toxic effects of pollution such as significant changes in fish behavior or external appearance may be evident on cellular or tissue level.

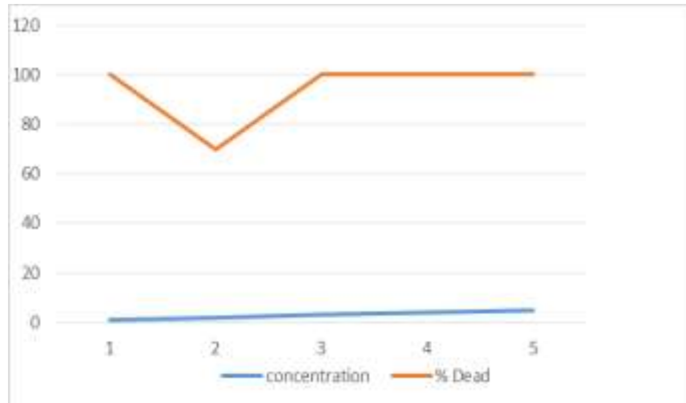

Figure 1. The regression line between the log concentration of copper sulphatea and probit kill of *Labeo rohita*.

The freshwaters plays a vital role in the production of fishes, prawns and crabs. But the sources of freshwater are ecologically worsening due to the untreated industrial discharges [57]. The entry of industrial effluents in the water bodies was harmful to fishes. The decline of fishes has been recorded in riverine systems due to receiving various pollutants. Abnormal physicochemical features of industrial discharge are accountable for death of fishes [58].

IV. CONCLUSION

In the aquatic ecosystem, some organisms were sensitive to high concentrations of heavy metals which cause harmful effects on them. It is helpful to find out the acceptable level of a toxicant in an ecosystem. The current study reported the 96-hours LC_{50} of copper sulphate (1.60 mg/L) for the *L. rohita*. An acute toxicity assessment can provide information about the state of an aquatic ecosystem and eventually assist in the formulation of policy recommendations for its preservation. It aids in the assessment of the harm that contaminants do to the environment and the development of water quality standards to save aquatic life.

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V. REFERENCES

[1] S. Chattopadhyay, S. S. Das, S. De. K. Chandra, M. Mishra, B. R. Sarkar and S. Ganguly, "Synthesis and evaluation of 99mTc-moxifloxacin, a potential infection specific imaging agent," *Applied Radiation and Isotopes*, vol. 68, no. 2, pp. 314–316, 2010.

[2] J. Kim and J. Kang, "Understanding the Toxicological Effects of Heavy Metals," *Environmental Science and Pollution Research*, vol. 24, no. 10, pp. 8201–8210, 2017.

[3] J. E. Fergusson-editor. "The Heavy Elements: Chemistry, Environmental Impact and Health Effects," *Oxford: Pergamon Press,* 1990.

[4] M. Tüzen, "Determination of heavy metals in soil, mushroom and plant samples by atomic absorption spectrometry,''*Microchemical Journal*, vol. 74, no. 3, pp. 289–297, 2003.

[5] C. R. Fernandez, R. O. Munoz and C. Camara, "Coupling pervaporation to AAS for inorganic and organic mercury determination A new approach to speciation of Hg in environmental samples,'' *Fresenius' journal of analytical chemistry*, vol. 371, pp. 1124–1129, 2001.

[6] R. K. Mudgal, R. Shankar, P. Talib and T. Raj, "Modelling the barriers of green supply chain practices: an Indian perspective,'' *International Journal of Logistics Systems and Management*, vol. 7, no. 1, pp. 8–1107, 2010.

[7] A. Yilmaz, O. Javed and M. Shah, "Object tracking: A survey,'' *Acm computing surveys (CSUR)*, vol. 38, no.4, pp. 13, 2006.

[8] G. M. Barton and R. Medzhitov, "Toll-like receptor signaling pathways'', *Science*, vol. 300, no. 5625, pp. 1524–1525, 2003.

[9] D. E. Kime, "The effects of pollution on reproduction in fish,'' *Reviews in fish biology and fisheries*, vol. 5, pp. 52–95, 1995.

[10] D. Purves and R. D. Hadley, "Changes in the dendritic branching of adult mammalian neurones revealed by repeated imaging in situ,'' *Nature*, vol. 315, no. 6018, pp. 404–406, 1985.

[11] C. Novotny, K. Svobodova, P. Erbanova, T. Cajthaml, A. Kasinath, E. Lang and V. sasek, "Ligninolytic fungi in bioremediation: extracellular enzyme production and degradation rate,'' *Soil Biology and Biochemistry*, vol. 36, no. 10, pp. 1545–1551, 2004.

[12] E. O. Farombi, O. A. Adelowo and Y. R. Ajimoko, "Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (Clariasgariepinus) from Nigeria Ogun River,'' *International journal of environmental research and public health*, vol. 4, no. 2, pp. 158–165, 2007.

[13] J. C. Akan, F. I. Abdulrahman , G. A. Dimari and V. O. Ogugbuaja, "Physicochemical determination of pollutants in wastewater and vegetable samples along the Jakara wastewater channel in Kano Metropolis, Kano State, Nigeria,'' *European Journal of Scientific Research*, vol. 23, no. 2, pp. 122–133, 2008.

[14] FAO, "Food and agriculture organization of the united nations," Rome, Italy, 2016.

[15] R. Aydın and K. Koprucu, "Acute toxicity of diazinon on the common carp (Cyprinuscarpio L.) embryos and larvae,'' *Pesticide biochemistry and physiology*, vol. 82, no. 3, pp. 220–225, 2005.

[16] J. Zaki and W. C. Williams, "Interpersonal emotion regulation,'' *Emotion*, vol. 13, no. 5, pp. 803, 2013.

[17] M. A. Rauf and S.S. Ashraf, "Fundamental principles and application of heterogeneous photocatalytic degradation of dyes in solution,'' *Chemical engineering journal*, vol. 151, no. 1-3, pp. 10–18, 2009.

[18] S. Hayat, B. Ali and A. Ahmad, "Salicylic acid: biosynthesis, metabolism and physiological role in plants,'' *Salicylic acid: A plant hormone*, pp. 1–14, 2007.

[19] A. M. Yousafzai, A. R. Khan and A. R. Shakoori, "Trace metal accumulation in the liver of an endangered South Asian fresh water fish dwelling in sub-lethal pollution,'' *Pakistan Journal of Zoology*, vol. 41, no. 1, 2009.

[20] F. E. Olaifa, A. K. Olaifa, A. A. Adelaja and A. G. Owolabi, "Heavy metal contamination of Clariasgariepinus from a lake and fish farm in Ibadan, Nigeria,'' *African Journal of Biomedical Research*, vol. 7, no. 3, 2004.

[21] A. S. Abdel Baki, M. A. Dkhil and S. Al Quraishy, "Bioaccumulation of some heavy metals in tilapia fish relevant to their concentration in water and sediment of WadiHanifah, Saudi Arabia,'' *African Journal of Biotechnology*, vol. 10, no. 13, pp. 2541–2547, 2011.

[22] A. A. Idriss and A.K. Ahmad, "Heavy metal concentrations in fishes from Juru River, estimation of the health risk,'' *Bulletin of environmental contamination and toxicology*, vol. 94, pp. 204–208. 2015.

[23] M. M. Authman, M. S. Zaki, E. A. Khallaf and H. H. Abbas, "Use of fish as bio-indicator of the effects of heavy metals pollution,'' *Journal of Aquaculture Research & Development*, vol. 6, no.4, pp. 1–13, 2015.

[24] H. P. Linder, "On areas of endemism, with an example from the African Restionaceae,''*Systematic biology*, vol. 50, no. 6, pp. 892–912, 2001.

[25] ATSDR and A.H. Hall, "Chronic arsenic poisoning,'' *Toxicology letters*, vol. 128, no. 1-3, pp. 69–72, 1990.

[26] R. Nakamura, W. Baumjohann, B. Klecker, Y. Bogdanova A. Balogh, H. Reme and A. Runov, "Motion of the dipolarization front during a flow burst event observed by Cluster,'' *Geophysical research letters*, vol. 29, no. 20, pp. 3–1, 2002.

[27] X. J. Stouthart, J. L. Haans, R. A. Lock and S. E. W. Bonga, "Effects of water pH on copper toxicity to early life stages of the common carp (Cyprinuscarpio),'' *Environmental Toxicology and Chemistry: An International Journal*, vol. 15, no. 3, pp. 376–383, 1996.

[28] E. O. Farombi, O. A. Adelowo and Y. R. Ajimoko, "Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African catfish (Clariasgariepinus) from Nigeria Ogun River,'' *International Journal of Environmental Research and Public Health*, vol. 4, no. 2, pp. 158–165, 2007.

[29] M. I. E. Halmi, K. K. Ahamad, M. Y. Shukor, M. H. Wasoh, A. A. Rachman, M. K. Sabullah, S. A. Ahmad and M. A. Syed, "Mathematical modelling of the degradation kinetics of Bacillus cereus grown on phenol,'' *Journal of Environmental Bioremediation and Toxicology*, vol. 2, no. 2, pp. 1–8, 2014.

[30] Solomon and Susan "Irreversible climate change due to carbon dioxide emissions," *Proceedings of the national academy of sciences*, vol. 106, no. 6, pp. 1704–1709, 2009.

[31] Annabi, Ali, S. Khaled and M. Imed,"Cadmium: bioaccumulation, histopathology and detoxifying mechanisms in fish," *American Journal of Research Communication*, vol. 62, pp. 1–4.

[32] N. Kumar, A. S. Zaw, H. E. Khine, K. Maharajan, K. L. Wai, B. Tan, S. Mastura and R. Goy, "Blood loss and transfusion requirements in metastatic spinal tumor surgery: evaluation of influencing factors," *Annals of surgical oncology*, vol. 23, pp. 2079–2086, 2016.

[33] R. R. Pandey, T. Mondal, F. Mohammad, S. Enroth, L. Redrup, J. Komorowski, T. Nagano, D. Mancini D. Nardo and C. Kanduri , "Kcnq1ot1 antisense noncoding RNA mediates lineage-specific transcriptional silencing through chromatin-level regulation," *Molecular cell*, vol. 32, no. 2, pp. 232–246, 2008.

[34] R. O. Vander, J. Beyer and N. P. Vermeulen, "Fish bioaccumulation and biomarkers in environmental risk assessment: a review," *Environmental toxicology and pharmacology*, Vol. 13, no. 2, pp. 57–149, 2003.

[35] K. Kumari and P. H. Weigel, "Molecular cloning, expression, and characterization of the authentic hyaluronan synthase from group C Streptococcus equisimilis," *Journal of Biological Chemistry*, vol. 272, no. 51, pp. 32539–32546, 1997.

[36] Eisler and Ronald, "Cadmium hazards to fish, wildlife, and invertebrates: a synoptic review,"*Fish and Wildlife Service, US Department of Interior* no. 2. 1985.

[37] L. Jarup, "Hazards of heavy metal contamination," *British medical bulletin*, vol. 68, no.1, pp. 167–182, 2003.

[38] M. I Castro Gonzalez and M. Mendez Armenta, "Heavy metals: Implications associated to fish consumption," *Environmental toxicology and pharmacology*, vol. 26, no. 3, pp. 263–271, 2008.

[39] A. P. H. A Standard, "Methods for the Examination of Water and Wastewater,'' Washington DC, no. 23 *American Public Health Association*, 2017.

[40] M. A. Hamilton, R. C. Russo and R. V Thurston, "Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays," *Environmental science and technology*, Vol. 11, no. 7, pp. 714–719, 1977.

[41] K. Kumari and P. H. Weigel, "Molecular cloning, expression, and characterization of the authentic hyaluronan synthase from group C Streptococcus equisimilis," *Journal of Biological Chemistry*, vol. 272, no. 51, pp. 32539–32546, 1997.

[42] M. H. Depledge and P. S. Rainbow, "Models of regulation and accumulation of trace metals in marine invertebrates," *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology*, vol. 97, no. 1, pp. 1–7, 1990.

[43] M. C. Newman, "Regression analysis of log-transformed data: Statistical bias and its correction," *Environmental Toxicology and Chemistry: An International Journal*, vol. 12, no. 6, pp. 1129–1133, 1993.

[44] A. M. Z. Boisen, J. Amstrup, I. Novak and M. Grosell, "Sodium and chloride transport in soft water and hard water acclimated zebrafish (*Daniorerio*),'' *Biochimica et BiophysicaActa (BBA)-Biomembranes*, vol. 1618, no. 2, pp. 207–218, 2003.

[45] J. Yakoob, M. R. Khan, Z. Abbas, W. Jafri, R. Azmi, Z. Ahmad, S. Naeem and L. Lubbad, "Helicobacter pylori: association with gall bladder disorders in Pakistan,"*British journal of biomedical science*, vol. 68, no. 2, pp. 59, 2011.

[46] A. K. Mishra and B. Mohanty, "Acute toxicity impacts of hexavalent chromium on behavior and histopathology of gill, kidney and liver of the freshwater fish,Channa punctatus (Bloch),"*Environmental Toxicology and Pharmacology*, vol. 26, no. 2, pp. 136–141, 2008.

[47] H. De Boeck and P. Opdecam, "Posteromedial Tibial Plateau Fractures: Operative Treatment by Posterior Approach,*Clinical Orthopaedics and Related Research (1976-2007)*, vol. 320, pp. 125–128, 1995.

[48] M. Abdullah Al Wadud, M. H. Kabir, M. A. A. Dewan and O. A. Chae, "dynamic histogram equalization for image contrast enhancement," *IEEE transactions on consumer electronics*, vol. 53, no. 2, pp. 593-600, 2007.

[49] F. Afridi, M. Bishnu and K. Mahajan, "Gender and mechanization: Evidence from Indian agriculture," *American Journal of Agricultural Economics*, vol. 105, no .1, pp. 52–75, 2023.

[50] F. Abdullah, T. Hassan and S. Mohamad, "Investigation of performance of Malaysian Islamic unit trust funds: Comparison with conventional unit trust funds,'' *Managerial Finance*, vol. *33,* no. 2, pp. 142–153, 2007.

[51] R. M. Dilshad and M. I. Latif," Focus group interview as a tool for qualitative research: An analysis,'' *Pakistan Journal of Social Sciences*, vol. *33,* no. 1, pp. 191–198, 2013.

[52] P. A. Gunatillake, R. Adhikari and N. Gadegaard, "Biodegradable synthetic polymers for tissue engineering,''*Eur Cell Mater*, vol. *5,* no. 1, pp. 1–16, 2003.

[53] M. T. Javed, N. Irfan and B.M. Gibbs, "Control of combustion-generated nitrogen oxides by selective non-catalytic reduction,'' *Journal of environmental management*, vol. 83, no. 3, pp. 251–289, 2007.

[54] N. Hamed, G. Esmail, A. Alireza, R. I. Mohammad and T. Vahid, "Effect of lead nitrate on survival rate and chronic exposure on growth performance of grass carp (Ctenopharyngodonidella)'', *Journal of Toxicology and Environmental Health Sciences*, vol. *4,* no.10, pp. 171–174, 2012.

[55] K. K. Das and S. K. Banerjee, "Cadmium toxicity in fishes,''*Hydrobiologia*, Vol. *75,* pp.117–121, 1980.

[56] S. A. Tomlins, D. R. Rhodes, S. Perner, S. M. Dhanasekaran, R. Mehra, X. W. Sun, S. Varambally, X. Cao, J. Tchinda, R. Kuefer and C. Lee, "Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer,'' *Science*, vol. 310, no. 5748, pp. 644–648, 2005.

[57] X. Thingran, "Industrial Effluents and Their Impact on Freshwater Ecosystems." Journal of Aquatic Ecology, vol. 8, no. 3, pp. 250–265, 1974.

[58] R. M. Heisey, J. Huang, S. K. Mishra, J. E. Keller, J. R., Miller, A. R. Putnam, and T. D. D'Silva, "Production of valinomycin, an insecticidal antibiotic, by Streptomyces griseus var. flexipertum,''*Journal of agricultural and food chemistry*, vol. 36, no. 6, pp. 1283–1286, 1988.

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