Journal of Scientific Research and Reports

Volume 30, Issue 6, Page 333-340, 2024; Article no.JSRR.116637
ISSN: 2320-0227

Physiological Changes in Nickel-exposed Nile Tilapia *Oreochromis niloticus* during Exposure and Recovery Periods

K. U., Sheethal [a], Prakash Nadoor [b], S. R. Somashekara [a], U. A. Suryawanshi [c], K. R. Amogha [a], P. A. Telvekar [c], S. T. Shelke [c] and J. G. K. Pathan [c*]

[a] College of Fisheries, Mangaluru, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, India.
[b] Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka, India.
[c] College of Fishery Science, Nagpur Maharashtra Animal and Fisheries Sciences University, Nagpur, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JSRR/2024/v30i62048

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/116637

Original Research Article

Received: 28/02/2024
Accepted: 02/05/2024
Published: 07/05/2024

ABSTRACT

Heavy metals are a significant problem in aquatic ecosystems as they are toxic and tend to accumulate, immediately affecting fish physiology. The present investigation was carried out to evaluate the sub-lethality of Nickel chloride on fingerlings of Nile tilapia, *Oreochromis niloticus* on long-term exposure to it is below safe concentrations1/fifth (9.39 ppm) and 1/10th (4.69 ppm). The physiological studies revealed a significant reduction in Oxygen Consumption Rate (OCR),

**Corresponding author: E-mail: drjgkpathan@gmail.com;*

Ammonia-N Excretion Rate (AER), Oxygen Rate (O: R) and Food Consumption Rate (FCR) and behavioural changes during accumulation and depuration phases. Limited recovery was obtained in all the physiological parameters after depuration for 28 days from the end of the accumulation period.

Keywords: Nickel; Oreochromis niloticus; physiology; exposure; recovery; metallic contaminants.

1. INTRODUCTION

The integrity of the aquatic environment has been threatened severely by a broad spectrum of contaminants such as metals, pesticides, chemicals, and industrial discharge [1, 2]. Given their capacity for bioaccumulation and potential toxicity, metallic contaminants are a focal point of concern within aquatic environments [3,4]. Heavy metals are discharged into the environment through various sources, including domestic, industrial, and other anthropogenic activities that may contaminate the natural aquatic system extensively [5,6]. Toxic effects of metals on organisms through accumulation in different organs, i.e. gill, liver, kidney, muscle, spleen, and brain [7]. Nickel is the dominant chemical species pollutant that occurs in natural waters [8-10]. It does not break down in the environment and accumulates after exposure to low levels in aquatic organisms. Due to the persistence and bioaccumulation of Nickel, its compounds produce immediate and prolonged harmful effects on aquatic biota [11]. Fish physiology is an integral part of aquatic toxicology, and pollutants at sub-lethal concentrations can be considered an exciting environmental variable to which a fish will physiologically respond [12]. The heavy metals alter the fish's physiological parameters by posing a negative impact [13]. To understand the toxicity mechanism of toxicant, it is important to know how a toxicant can enter into the organism, how it interacts with target molecules and exerts its effects and how the organism shows physiological responses, including behavioural changes, oxygen consumption rate, ammonia-N excretion rate and feeding activity with exposure to toxicant which gives a better understanding of fundamental physiological processes [14,15]. Hence, efforts were undertaken to understand the knowledge of physiological changes in *O. niloticus* exposed to sub-lethal concentrations of Nickel chloride.

2. MATERIALS AND METHODS

A lethal toxicity study was carried out by following the standard guidelines [16] to determine the lethal (LC_{50}) level of Nickel Chloride using the static bioassay method. Merck grade Nickel chloride hexa hydrate ($NiCl_2 \cdot 6H_2O$) is a toxicant. Uniform sizes of fish fingerlings of Nile tilapia, *Oreochromis niloticus* with length and weight range 8.5 to 10 cm and 9.5 to 12g were used to assess the lethal concentration of the toxicant. Water quality parameters were analysed following the methods mentioned in APHA [16] and found as follows: water temperature: 24±2°C, pH: 7.1±0.2 at 24°C, dissolved oxygen: 9.3±0.8 mg/L, carbon dioxide: 6.3±0.4mg/L, total hardness: 23.4±3.4 mg as $CaCO_3$/L, phosphate: 0.39±0.002 µg/L and salinity: nil. All the tests were performed in triplicates and appropriate controls with 6 No. of fish in each replicate for 96 hours, and mortality was recorded at intervals every six hours. The LC_{50} values were estimated by the probit analysis method [17].

2.1 Physiological Study

Physiological variables such as oxygen consumption rate, ammonia-N excretion rate, Oxygen: Nitrogen (O: N) ratio, food consumption rate, and behavioural changes were studied. During the study period, the water quality parameters such as temperature, pH, DO, Alkalinity, hardness and nutrients (Ammonia and nitrite) were assessed following standardised procedures [16].

Physiological parameters such as oxygen consumption by fish were determined by Zhen et al. [18] method. The ammonia excretion rate was estimated every 24 hours by the phenol hypochlorite method [19]. Absorbance was measured spectrophotometrically at 640 nm. The Oxygen: Nitrogen ratios were calculated from the oxygen consumption and ammonia excretion rates in *O. niloticus*. The Oxygen to Nitrogen ratio was determined by comparing the oxygen consumed to the nitrogen excreted within the specified interval [20, 21]. The fingerlings were fed daily with prepared pelleted feed to determine the food consumption rate. The leftover feed was siphoned out after 60 minutes. The collected feed was dried overnight at 600C in a hot air oven and weighed to compare mean food consumption as per the methods of Broeck et al. [22].

2.2 Statistical Analysis

The LC50 value was estimated using the probit analysis method [17]. Physiological changes were tested using one-way ANOVA (analysis of variance). Post hoc tests were carried out using Duncan's multiple-range test comparison procedure. All the statistical analyses were performed via the SPSS 20.0 version.

3. RESULTS AND DISCUSSION

3.1 Oxygen Consumption Rate

The changes in the oxygen consumption rate are a good index of the metabolic capacity of an organism to experience environmental stress [23]. It is apparent from the result that Nickel chloride influences oxygen consumption in fish. The normal respiratory metabolism can be altered because of its proximity to contaminated water, which decreases the oxygen-diffusing capacity of fish gills [24, 25]. During the current study, Nickel chloride significantly reduced the oxygen consumption rate of fingerlings of O. niloticus under sub-lethal concentrations (1/10th and 1/5th of LC50). It showed a gradual increase trend during the depuration period (Fig.1).

A decrease in oxygen consumption in fingerlings of *Tilapia mossambica* on exposure to cadmium [26, 27]**.** Their results indicate that the gills were the main target of pollutants and damaged the gills [28]. Also, Padmanabha et al. [29] observed a significant reduction in the rate of oxygen consumption in fish exposed to sub-lethal concentrations of cadmium (0.0022 ppm). Also, studies by Grinwis et al. [30] and Hartl et al. [31] have revealed that a decrease in the respiratory rate in sub-lethal concentrations was due to toxicant-induced stress on gills or membrane functions in the fish.

3.2 Ammonia-N Excretion Rate

The ammonia-n excretion rate indicates nitrogen balance and elucidates the influence of environmental and nutritional factors on protein metabolism in fish [32]. In freshwater fish, most nitrogenous end products stem from protein catabolism, with ammonia-N being a primary end product. The assessment of ammonia excretion contributes to determining the total energy production of the fish, reflecting the extent of protein catabolism [33]. In this investigation, ammonia- N excretion of *O. niloticus* decreased significantly during the accumulation period when subjected to one-tenth and one-fifth concentration of nickel chloride over 28 days, and the fingerlings of *O. niloticus* showed a gradual increase in the ammonia-N excretion rate when exposed to clean water (Fig.2). Parveen and Javed [34] observed an increase in ammonia excretion rate –N when *Catlacatla* was exposed to water-borne Copper for 90 days. In addition, Jisha et al. [35] recorded a significant increase in the ammonia quotient in Cadmium chloride-exposed tilapia, *O. mossambicus*, over seven days. Similarly, Padmanabha et al. [29] observed a significant decrease in ammonia excretion in the fingerlings of *O. mossambicus* subjected to a sub-lethal dose of Cadmium (0.0022 ppm). Further, they determined that the reduction of the ammonia-N excretion rate in both the sub-lethal concentrations was due to toxicants-induced stress, fish avoidance and the biotransformation process. Grosell et al*.* [36] recommended decreasing the ammonia excretion rate due to metal-induced stress on the excretory system in freshwater fish exposed to metal.

3.3 Oxygen: Nitrogen Ratio

Oxygen consumption by aquatic animals is a sensitive physiological process. Therefore, alteration in respiratory activity is measured as an indicator of the stress of animals exposed to heavy metals [37]. At the same time, ammonia excretion rates could indicate a higher reliance on proteins in stressed animals [20, 21]. Pillai and Diwan [38] highlighted that the ratios of oxygen consumption to ammonia-N excretion in atomic equivalents offer insights into alterations in energy substrate utilisation across different environmental conditions.

It is apparent from the results that during In this investigation, the nickel chloride has affected the Oxygen: Nitrogen ratio in fingerlings of Nile tilapia (*O. niloticus)* under sub-lethal concentrations by showing gradually decreased values in the O: N ratio compared to the control group gradually increased values during accumulation and depuration phases respectively (Fig.3). Padmanabha et al. [26] observed a significant increase in Oxygen: Nitrogen ratio in fingerling of *O. mossambicus* subjected to sub-lethal concentration of cadmium (0.0022 ppm) and Inhibition of oxygen consumption and increase ammonium excretion by Cd has been reported in *Litopenaeus vannamei* [39] because it diminze the efficiency of gaseous exchange.

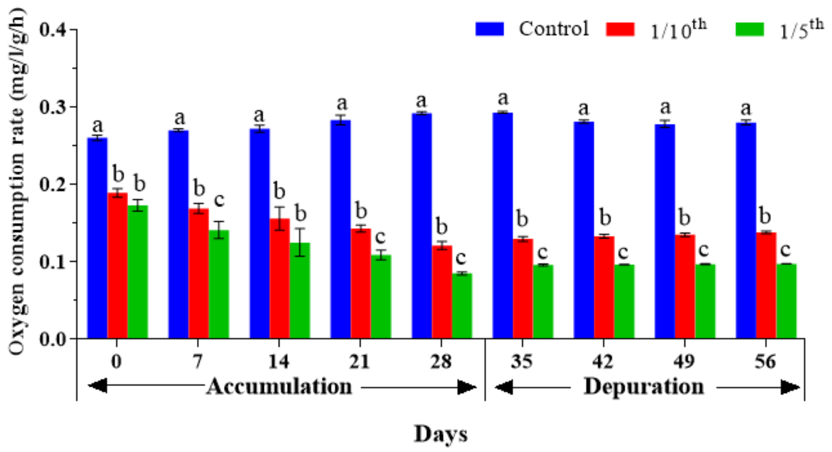

Fig. 1. Variation of the oxygen consumption rate (mg/l/g/h) in *Oreochromis niloticus* during accumulation and depuration phase on exposure to different sub-lethal concentrations of Nickel chloride. (*a,b,c significance difference between treatments)

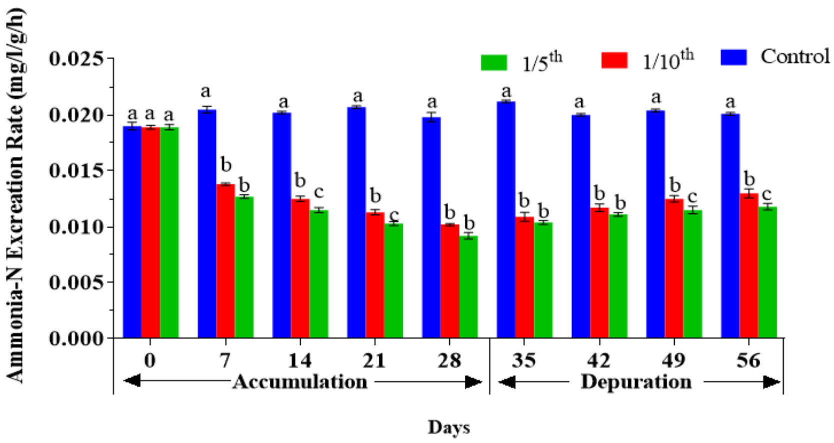

Fig. 2. Variation of the rate of ammonia-N excretion (mg/l/g/h) in *Oreochromis niloticus* during accumulation and depuration phase on exposure to different sub-lethal concentrations of Nickel chloride. (*a,b,c significance difference between treatments)

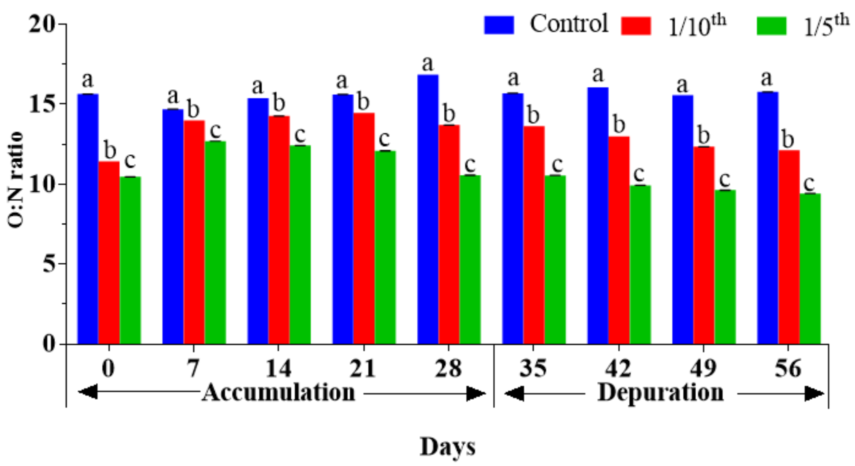

Fig. 3. Variation of the Oxygen: Nitrogen ratio rate in *Oreochromis niloticus* during accumulation and depuration phase on exposure to different sub-lethal concentrations of Nickel chloride. (*a,b,c significance difference between treatments)

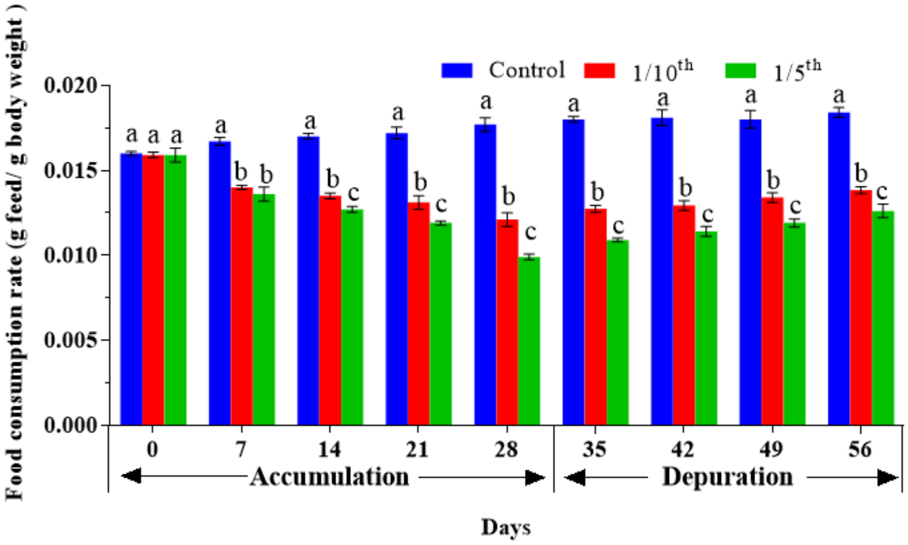

Fig. 4. Variation of the rate of Food consumption rate (g feed/ g body weight) in *Oreochromis niloticus* during accumulation and depuration phase on exposure to different sub-lethal concentrations of Nickel chloride. (*a,b,c significance difference between treatments)

Studies during the present investigation revealed that increased sub-lethal concentration of nickel chloride resulted in decreased respiration and decreased excretion in *Oreochromis niloticus*. Consequently, O: N ratios decreased with increased concentration of nickel chloride.

3.4 Food Consumption Rate

Changes in feeding behaviour are considered a sensitive indicator for detecting pollution due to metal nickel chloride. The results revealed that food consumption decreased significantly in the fishes exposed to nickel chloride. In the depuration period, it gradually increased (Fig.4).

The sub-lethal concentration of copper in water (0, 0.15, 0.3 and 0.5 ppm.) on the feed consumption of *O. niloticus* was studied [40]. Fish refused to accept the feed immediately after exposure and began taking it up after 4 hours compared with the control. The fish exhibited significantly reduced feed consumption when exposed to varying concentrations of copper in water compared to the control group. Further, they recommended that the lowered food intake of fish could be the effect of copper on the central nervous system. Similarly, the fingerlings of Nile tilapia (*O. niloticus*) exposed to nickel chloride consumed less food than the control group. Thus, heavy metals might disturb the development of locomotor abilities that might severely affect the feeding ability of fish.

The sub-lethal effect of concentrations of cadmium (1/10th and 1/5th, i.e. 17ppm, 34 ppm) significantly decreased the food consumption rate(feed consumption body wt.) in *O. mossambicus* over 48 hours [29]. Reduction in feeding behaviour under toxic environmental conditions might lower the energy costs of digestion [41]. The effects of three different sub-lethal concentrations (0.20 µM, 0.55 µM and 0.80 µM) of copper on food consumption in juveniles of common carp, C. carpio for 28 days was studied [22] and noticed a decrease in food consumption rate compared to control. The decreased food intake may be attributed to damage caused to taste receptors, as reported by Gerking, S. D. [42]. In this context, Heath [43] thinks that the fish subjected to long-term exposure to pollutants exhibited a reduction in appetite.

3.5 Behavioural Changes

Physiological changes are vital attributes that indicate the health condition of organisms. All behavioural modifications are observed as aquatic organisms serve as sensitive indicators of stress induced by environmental chemicals. Behavioural changes such as erratic swimming, restlessness and surfacing phenomena, and mucous secretion are attributed to fish and may protect against ecological contaminants [44, 45].

Throughout this study, the control group of fish exhibited natural behaviour, characterised by active feeding and heightened awareness of minor disturbances, demonstrated through coordinated movements. Nile tilapia (O. niloticus) exhibited disrupted shoaling behaviour in toxic media, localisation to the experimental tank's lower section and swimming independence. The described symptoms were accompanied by a loss of coordination among individuals and an increase in the occupied area of the control group, with sub-lethal concentrations indicating the early effects of nickel exposure in both.

Khunyakari et al.,[46] studied the conduct of fishes exposed to nickel, copper and zinc in *Poecilia reticulate*. They suggested that heavy metal exposure caused mucus-like discharge on gills, heightened excretion, anorexia, and escalated fin activity. A similar type of behavioural observation was recorded by Yaji et al*.* [47] in *Orechromis niloticus* treated with Cypermethrin. Janardhana Reddy and Reddy [48] noticed similar behavioural anomalies in the Cadmium exposed to *Catla catla,* showing respiratory rate impairment, skin irritation, and coughing provoked by the toxicants. The air gulping may help avoid contact with a toxic medium, and the surfacing phenomenon might demand elevated oxygen concentrations throughout the exposure period [49]. Loss of fish balance might be due to neurological impairment in the central nervous system, as evidenced by Cadmium's inhibition of the Acetylcholine enzyme [50].

4. CONCLUSION

Exposure to nickel chloride significantly affects various physiological parameters, including oxygen consumption, ammonia excretion, oxygen-nitrogen ratio, food consumption rate, and behavioural patterns, in Nile tilapia fingerlings. These findings highlight the detrimental effects of heavy metal pollution on fish health and behaviour, underscoring the importance of environmental monitoring and conservation efforts to mitigate such impacts on aquatic ecosystems.

ACKNOWLEDGEMENT

The authors thank the Dean (Fisheries) for their constant encouragement and valuable suggestions during my research tenure and express their sincere gratitude to the Dean, College of Fishery, Mangaluru, Karnataka Veterinary University, Bidar, for support by providing sufficient facilities during the study period.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lee SJ, Mamun M, Atique U, An KG. Fish tissue contamination with organic pollutants and heavy metals:Link between land use and ecological health. Water. 2023;15(10):1845.
2. Vinodhini R, Narayanan M. Bioaccumulation of heavy metals in organs of freshwater fish *Cyprinius carpio*. Int. J. Environ. Sci. Tech. 2008;5(2):179-182.
3. Kumar V, Umesh M, Shanmugam MK, Chakraborty P, Duhan L, Gummadi SN, Dasarahally Huligowda LK. A retrospection on mercury contamination, bioaccumulation, and toxicity in diverse environments: current insights and future prospects. Sustainability. 2023;15(18):132 92.
4. Censi P, Spoto SE, Saiano F, Sprovieri M, Mazzola S, Nardone G, Di Geronimo SI, Punturo R, Ottonello D. Heavy metals in coastal water systems. A case study from the northwestern Gulf of Thailand. Chemosphere. 2006;64:1167-1176
5. Zhang P, Yang M, Lan J, Huang Y, Zhang J, Huang S, Ru J. Water quality degradation due to heavy metal contamination: Health impacts and eco-friendly approaches for heavy metal remediation. Toxics. 2023;11(10):828.
6. Velez D, Montoro R, Arsenic speciation in manufactured seafood products: A review. J. food Protect. 1998;9(61):1240-1245.
7. MacFarlane GB, Burchettt MD. Cellular distribution of Cu, Pb, and Zn in the Grey Mangrove *Avicemnia marina* (Forsk.). Vierh Aquatic Botanic. 2000;68:45-59
8. US, EPA. Ambient water quality criteria for nickel. EPA440/5-80-060. Office of Water Regulations and Standards. Washington, DC; 1980.
9. US, EPA. Ambient water quality criteria for nickel – EPA-440/5-86-004. Office of Water Regulations and Standards. Washington, DC. 1986.
10. Eisler R. Nickel hazards to fish, wildlife and invertebrates: A synoptic review. Biological

Science Report USGS/BRD/BSR-1998-0001, Laurel, MD. 1998.

11. Vieira LR, Gravato C, Soares AMVM, Morgado F, Guilhermino L. Acute effects of copper and mercury on the estuarine fish *Pomatoschistus microps*: Linking biomarkers to behaviour. Chemosphere. 2009;76(10):1416–1427
12. Allen Heath G. Water pollution and fish physiology. Edition 2nd. Lewis publishers. 1995; 6-7
13. Viella S, Ingrossi L, Lionetto M, Schettino T, Zonno V, Stroelli C. Effect of cadmium and zinc on the Na/H exchanger on the brush border membrane vesicles isolated from eel kidney triangular cells. Aquat.Toxicol. 199948:25-36
14. Lushchak VI, Matviishyn TM, Husak VV, Storey JM, Storey KB. Pesticide toxicity: a mechanistic approach. EXCLI J. 2018;8 (17):1101-1136.
15. Sheehan PJ, Miller DR, Butler GC, Bourdea UP. Effects of pollutants at ecosystem level. Scope 22. Chichester: Wiley &Sons Ltd; 1984.
16. ANON, APHA. Standard methods for the examination of water and wastewater. *21stEdn.,* Washington, D.C. 2005.
17. Finney DJ. Probit Analysis. 1st Edn., Cambridge University Press, Cambridge, 1971ISBN-10: 0521135907, 272.
18. Zhen Y, Aili J, Changhai W. Oxygen consumption, ammonia excretion, and filtration rate of the marine bivalve Mytilus edulis exposed to methamidophos and omethoate. Marine and Freshwater Behaviour and Physiology. 2010;43(4): 243-255.
19. Solorzano L. Determination of ammonia in natural waters by the phenol hypochlorite method*.* Limnol. Oceanogr. 1969;14:799
20. Ramírez JFP, Amanajás RD, Val AL. Ammonia Increases the Stress of the Amazonian Giant Arapaima gigas in a Climate Change Scenario. Animals. 2023; 13(12):1977.
21. Bayne BL, Brown DA, Burns K, Dixon DR, Ivanovici A, Livingstone DR, Lowe DM, Moore MN, Stebbing ARD, Widdows J. The effects of stress and pollution on marine animals. Praeger Scientific, New York. 1958;384
22. Broeck GD, Vlaeminck A, Blust R. Effects of sub-lethal copper exposure on copper accumulation, food consumption, growth, energy stores, and nucleic acid content in common carp. Arch. Environ. Contam. Toxicol. 1997;33:415-422
23. Jadhav SS, Surve PR, Gundile MG. Impact of mercuric nitrate on the oxygen consumption of freshwater crab, Barytelphusa Guerini. Ecology Environment and Conservation. 2006;12 (4):749.
24. Gokul T, Kumar KR, Prema P, Arun A, Balaji P, Faggio C. Particulate pollution and its toxicity to fish: An overview. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 2023;109646.
25. Khoshnood Zahra. Effects of environmental pollution on fish: A short review. Transylv. Rev. Syst. Ecol. Res. 2017;19(1):49-60.
26. Padmanabha A. Physiological and Biochemical Responses in Tilapia *(Oreochromis mossambicus)* Exposed to Cadmium and Chlorpyrifos (Doctoral dissertation, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar); 2015.
27. Usharani A, Ramamurthi R. Effects of sublethal concentration of cadmium on oxidative metabolism in the freshwater teleost, *Tilapia mossambica*. *Indian J. Comp.* Anim. Physiol. 1987;5 (2):71-80.
28. Al-Yakoob S, Bou-Oluyan AH. and Bahlool, M. Trace metals in gills of fish from the Arabian Gulf. Bull. Environ. Contam. Toxicol. 1994; 53(5):718-752.
29. Padmanabha A, Reddy HRV, Muttappa Khavi, Prabhudeva KN, Rajanna KB, Chethan N. Acute effects of chlorpyrifos on oxygen consumption and food consumption of freshwater fish, *Oreochromis mossambicus* (Peters). Inter. J .of Recent Scien. Resea. 2015;6:3380-3384
30. Grinwis GCM, Boonstraa, Van Den Brandhof FJ, Dormans J, Engelsma M, Kuiper RV, Vanloveren H, Wester PW, Vaal MA, Vethaak AD, Vos JG. Short-term bis (tri-n-butyltin) oxide toxicity in flounder (*Platichthysflesus*). Pathology and immune function. Aquat. Toxicol.1998;42:15-36.
31. Hartl MGJ, Hutchinson S, Hawkins L. Organotin and osmoregulation: Quantifying the effects of environmental concentrations of sediment-associated TBT and TPhT on the freshwater adapted European flounder, *Platichthys flesus*. J. Exp. Mar. Biol. Ecol. 2001;256;267-78.

32. Patricia AW, Land MD. Urea Production and Transport in Teleost Fishes,Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology.1998;119(1):47-54.
33. Deboeck G, Desmet H, Blust R. The effect of sub-lethal levels of copper on oxygen consumption and ammonia excretion in the common carp *Cyprinus carpio* (L.). Aquat. Toxicol. 1995;32:127-141
34. Parveen A, Javed M. Effect of water-borne copper on the growth performance of fish *Catla catla*. Int. J. Agric. Bio.2010;12:950-952
35. Jisha J, Hari Sankar HSA, Smitha BV, Aniladevi Kunjamma KPA, Remya V. Babu Philip. Cadmium ion-induced changes in the protein catabolism of *Oreochromis mossambicus.* International Journal of Scientific and Research Publications. 2013;3:1-8
36. Grosell M, Nielsen C, Bianchini A. Sodium turnover rate determine sensitivity to acute copper and silver exposure in freshwater animals. Comp. Biochem., Physiol. C. 2002;133:287–303.
37. Jadhav SS, Shinde VD, Sirsat D,. Katore BP, Ambore NE. Impact of Mercuric Nitrate on the Oxygen Consumption of Fresh Water Crab. *Barytelphus aguerini.* Rec Res Sci Tech. 2011;3:50-51.
38. Pillai Bindu R, Diwan AD. Effects of acute salinity stress on oxygen consumption and ammonia excretion rates of the marine shrimp *Metapenaeus monoceros*. Journal of Crustacean Biology. 2002;22(1):45-52.
39. Xuan R, Wu H, Lin C, Ma D, Li Y, Xu T, Wang L. Oxygen consumption and metabolic responses of freshwater crab *Sinopotamon henanense* to acute and sub-chronic cadmium exposure. Ecotoxicology and Environmental Safety. 2013;89:29-35.
40. Ali A, Al-Ogaily S M, Al-Asgah NA, Gropp J. Effect of sub-lethal concentrations of copper on the growth performance of *Oreochromis niloticus*. Jour. of Applied Ichthyology. 2003;19(4):183-188.
41. Halappa R, David M. Behavioural responses of the freshwater fish, *Cyprinus carpio* (Linnaeus) following sublethal exposure to chloropyrifos. Turk. J. Fish. Aquat. Sci. 2009;9:233-238.
42. Gerking SD. Feeding ecology of fish. Elsevier; 2014.
43. Heath AG. Water Pollution and Fish Physiology. CRC Press, Florida, USA. 1987:245.
44. Sharma M. Behavioural responses in effect to chemical stress in fish: A review. International Journal of Fisheries and Aquatic Studies. 2019;7(1):01-0
45. Svecevièus G. Avoidance response of rainbow trout *Oncorhynchus mykiss* to heavy metal model mixtures. A comparison with acute toxicity tests. *Bull. Environ. Contam.* Toxicol. 2001; 67:680-687
46. Khunyakari RP, Vrushali T, Sharma RN, Tare V. Effects of some Trace Heavy Metals on *Poecilia reticulate*, J. of Envi. Bio. 2001;22(2):141- 144.
47. Yaji AJ, Auta J, Oniye SJ, Adakole JA. Usman JI. Effects of Cypermethrin on behaviour and biochemical indices of freshwater fish *Oreochromis niloticus*. EJEAFChe. 2011;10(2):1927-1934
48. Janardhana Reddy S, Reddy DC. Impact of cadmium toxicity on behavioural and haematological biomarkers of freshwater fish, *Catla catla.* Int. J. Bioassays. 2013;02 (09):1199-1204.
49. Katja Schmidt, Georg BO, Staaks Stephan Pflugmacher, Christian EW Steinberg. Impact of PCB mixture (Aroclor 1254) and TBT and a mixture of both on swimming behaviour, body growth and enzymatic biotransformation activities (GST) of young carp (*Cyprinus carpio*). Aquatic Toxicology. 2005;71(1):49-59.
50. Patro L. Toxicological effects of cadmium chloride on Acetylcholinesterase activity of freshwater fish, *Oreochromis mossambicus* Peters. Asian J. Exp. Sci. 2006;20(1):171-180

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/116637