



Nickel induced histopathological alterations in the Female Reproductive Organs of freshwater fish, *Channa gachua* (Ham.)

Sujata Kawade

Department of Zoology,
Shri Shivaji Science College,
Amravati, Maharashtra.

Abstract

Heavy metals, on entering aquatic environment causes histopathological disturbances in the fish. The present study deals with the toxic effect of heavy metal - Nickel (Ni) as NiSO₄ on the Female Reproductive Organs – Ovary of fresh water fish, *Channa gachua*. Ovary was examined in the 96 hours LC₅₀ acute test. Histopathological examination of Ovary revealed marked pathological changes like rupture of ovigerous lamellae, rupture, degeneration and absorption of growing oocytes, degeneration and necrosis of connective tissue, formation of atretic follicles with suppression of oocyte maturation, rupture of blood vessels, thus affecting the process of reproduction in the female fishes.

Key words: Nickel, Ovary, histopathology, *Channa gachua*

INTRODUCTION:

Water is an essential resource for human survival. The global use of freshwater has increased many folds in the past years due to population explosion, urbanization, industrialization and agricultural production causing increased water consumption. Because of this, water quality is facing severe challenges resulting in degradation and pollution of the aquatic environment. This ultimately affects the aquatic fauna and flora, indirectly affecting the human health (Lin et al, 2022).

Many workers have reported the effect of pollutants on aquatic organisms. Shafir et al, 2007 reported short and long term toxicity of crude oil and oil dispersants on two soft and hard coral species at early life stages. Hughes et al, 2007 reported many coral reefs undergone phase shifts to alternate, degraded assemblages because of the combined effects of over-fishing, declining water quality, and the direct and indirect impacts of climate change. Environmental implications of plastic debris in marine settings causing entanglement, ingestion, smothering, hangers-on, hitch-hiking and alien invasions, as reported by Gregory, M R, 2009. Pesticide exposure can directly affect all levels of biological organization, including primary producers,

microorganisms, invertebrates, or fish reported by **Shefali et al, 2021**. Toxicity of herbicides in non-target organisms were reported by **Jurado et al, 2011**. Haematopoietic alterations were induced by carbaryl in *Clarias batrachus* (LINN) (**Patnaik & Patra, 2006**).

Of the various aquatic pollutants, heavy metals have a potential threat to aquatic organisms mainly fishes. They are non - biodegradable and tend to accumulate in the food chain. Heavy metals in fish mainly come from their diet, which are then passed from one trophic level to the other in the food chain (**Jamil et al, 2023**). Many workers have reported the bioaccumulation of heavy metals in aquatic organisms. **Sherly et al 2022** reported Bioaccumulation of heavy metals in edible tissue of crab (*Scylla serrata*) from an estuarine Ramsar site in Kerala, South India. **Vajargah et al, 2018** reported histopathological lesions and toxicity in common carp (*Cyprinus carpio* L. 1758) induced by copper nanoparticles. **Bibak et al, 2020** reported marine macro algae as a bio-indicator of heavy metal pollution in the marine environments, Persian Gulf. **Sattari et al, 2019** reported investigation of metal element concentrations in tissue of *Rutilus frisii* in the Southwest Caspian Sea. **Yalsuyi & Vajargah, 2017** reported acute toxicity of silver nanoparticles in Roach (*Rutilus rutilus*) and Goldfish (*Carassius auratus*). Effects of heavy metals Lead, Mercury, Cadmium, Copper, Zinc, Cadmium, Nickel on haemoglobin and haematocrit of fish haematology were studied by **Ahmed Et al, 2022**. **Ahmed et al, (2013)** reported Chromium (VI) induced acute toxicity and genotoxicity in freshwater stinging catfish, *Heteropneustes fossilis*. **Abdel-Warith et al, 2020** investigated the effects of sub-lethal lead nitrate and copper sulfate concentrations on haematological parameters during long-term exposure in Nile tilapia (*Oreochromis niloticus*). **Dutta and Dalal, 2008** studied the effect of endosulfan on the ovary of bluegill sunfish (*Lepomis macrochirus* sp). **Ghazaly, K. S. (1992)** reported sublethal effects of nickel on carbohydrate metabolism, blood and mineral contents of *Tilapia nilotica*. **Han et al, (2019)**. Reported the toxic effects of arsenic on growth, haematological parameters, and plasma components of starry flounder, *Platichthys stellatus*, at two water temperature conditions. **Hilmy et al, (1987)** studied Some physiological and biochemical indices of zinc toxicity in two freshwater fishes, *Clarias lazera* and *Tilapia zilli*. **Martinez et al, (2004)** studied acute morphological and physiological effects of lead in the neotropical fish *Prochilodus lineatus*.

Nickel is a highly toxic and nonessential heavy metal. Used in a range of industrial practices, production of stainless steel, Ni-Cd batteries, plating processes, refining ores, etc requires nickel. It is a nonbiodegradable chemical pollutant and a xenobiotic of the aquatic environment when present in higher concentrations. Fishes are chief organism of the aquatic system that can serve as biomarkers of aquatic pollution (**Kevin et al, 2017**). Heavy metals enter fish bodies through general body surface, consumption of polluted food, or through the gills, where they accumulate it in the body after absorption. Ultimately, this way heavy metals make their way into humans through the food chain.

Histopathological evaluation is an essential tool in pollution impact assessment to indicate the effect of toxicants on fish health (**Bernet et al, 1999**). The alterations in the histological structure may serve as an initial indicator of the toxicant's impact on fish health. It has been observed that when pollutants impact the aquatic environment, the first consequence is apparent

in the form of histological changes in fish. The study of histological changes due to the effect of pollutants in fishes is a suitable method to check the aquatic pollution (Yousif et al, (2016). The present study deals with the histopathological alterations in the Female reproductive Organs - Ovary of fish o exposure to heavy metal Nickel, and can be used as biomarker in various pollution monitoring programs.

MATERIALS AND METHODS:

Animal collection and maintenance:

Adult and live fish, *Channa gachua* were collected from the local market and brought to the laboratory. Healthy fishes, (Length-12-15 cms; Weight 50-56 gms) taken for the experiment were acclimatized in the glass aquaria for 15 days and fed with fish food. Water in the aquarium was replaced after every 24 hours.

Test chemical:

Stock solution of Nickel Sulphate (NiSO_4) was prepared by dissolving appropriate amount of NiSO_4 as Ni salt in distilled water.

Bioassay:

Fishes were divided in two groups- Control and Experimental. In experimental group, the fishes were exposed to Nickel for a period of 96 hours. The control group were also maintained, simultaneously. The fishes that survived at the end of exposure period, were sacrificed, dissected carefully to isolate the target organ ovary and fixed in the Bouins' fluid. The tissues were then proceeded for the Haematoxylin & Eosin (H & E) staining. The sections were examined under the light microscope (400X) and photographed.

RESULT:

Ovary of the controlled fish reported normal architecture showing the ovigerous lamellae, growing oocytes, connective tissue, blood vessels. (Fig 1)

In contrast to this, histopathological examination of Ovary of treated fish with lethal concentration of Nickel for 96 hrs at 150 ppm (LC_{50}), exhibited marked pathological changes like rupture of ovigerous lamellae, rupture and degeneration of growing oocytes, necrosis of connective tissue, formation of atretic follicles with suppression of oocyte maturation, rupture of blood vessels, thus affecting the process of oogenesis and reproduction in the female fishes. (Fig 2).

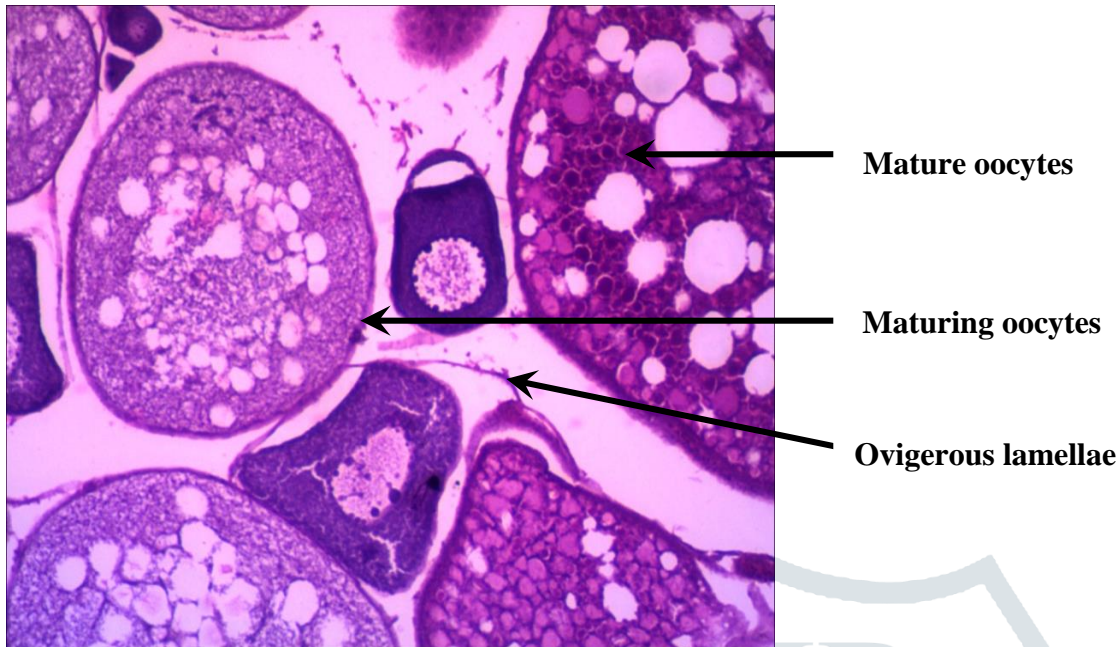


Fig 1: T. S. of Ovary of *Channa gachua* (Normal) (H/E) (400X)

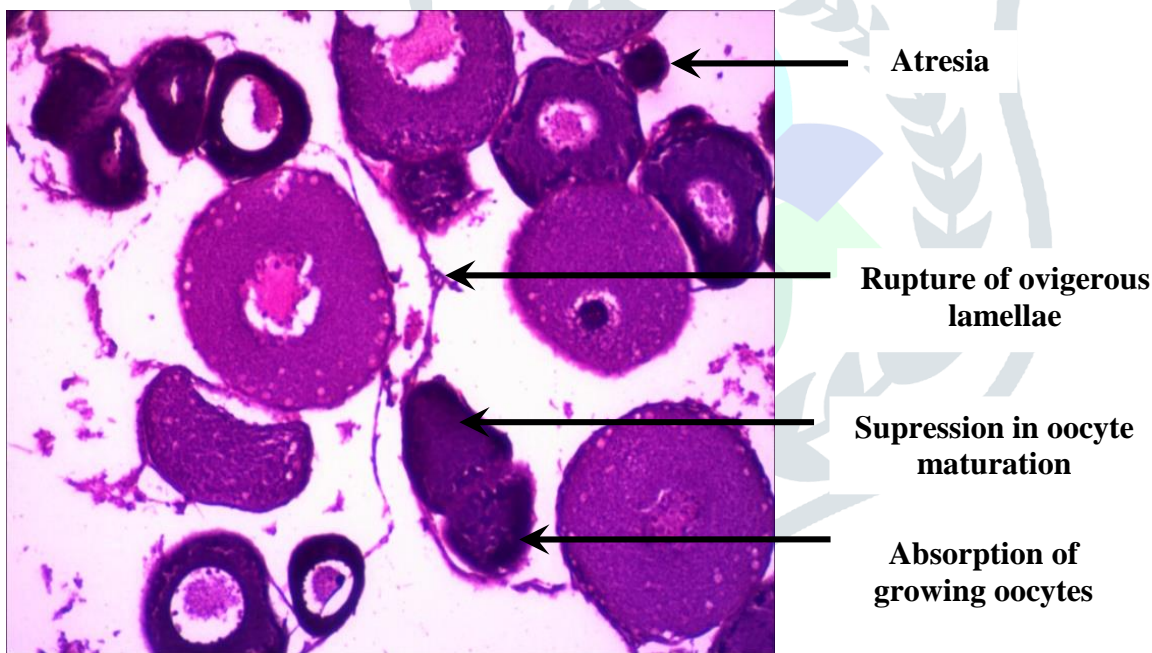


Fig 2: T.S. of Ovary of *Channa gachua* after exposure to heavy metal Nickel (Ni) as NiSO_4 at 150 ppm for a period of 96 hrs. (H/E) (400X)

DISCUSSION:

Water pollution has become a serious problem causing adverse impact on the sustainability of water resources, affecting the living organisms, It indirectly affects the health of the population and the economy, as well. This is mainly due to an increase in population activities and urban life (Lopes et al, 2022). Wastewater from industrial & residential zones make their way into the natural water bodies, thus deteriorating the quality of water bodies.

Ovary is a female reproductive organ, where eggs are produced and stored and are the site of reproduction in fishes. In the control fish, sections of Ovary reported normal architecture showing the ovigerous lamellae, growing oocytes, connective tissue, blood vessels. (Fig 1). However, histopathological examination of ovary of treated fish with lethal concentration of Nickel for 96 hrs at 150 ppm (LC₅₀), exhibited marked pathological changes like rupture of ovigerous lamellae, rupture and degeneration of growing oocytes, necrosis of connective tissue, formation of atretic follicles with suppression of oocyte maturation, rupture of blood vessels, thus affecting the process of oogenesis and reproduction in the female fishes. (Fig 2). These investigations are in accordance with the study reported by **Bhat et al, 2022** showing toxic effect of heavy metals on ovarian deformities, apoptotic changes, oxidative stress, and steroid hormones in rainbow trout.

The cellular disintegration in ovary is due to accumulation of heavy metal nickel which in turn affect the process of oocyte maturation as well as reproductive mechanism in fish (**Taslina et al, 2022**). These histopathological changes in the ovary are due to metal ion-ovary tissue interaction which lead to degenerative changes resulting in altered metabolic activity (**Gupta et al, 2021**). In fishes, these interactions disturb homeostasis leading to an unstable internal environment (**Yamaguchi et al, 2007**).

The toxicity effects of heavy metals on Ovary has been studied by many workers. **Gárriz et al, 2019** reported effects of heavy metals identified in Chascomús shallow lake on the endocrine-reproductive axis of pejerrey fish (*Odontesthes bonariensis*). **Yan et al, 2020** reported individual and combined toxicogenetic effects of microplastics and heavy metals (Cd, Pb, and Zn) perturb gut microbiota homeostasis and gonadal development in marine medaka (*Oryzias melastigma*). **Forouhar et al, 2020** studied the effects of copper oxide nanoparticles (CuO-NPs) on parturition time, survival rate and reproductive success of guppy fish, *Poecilia reticulata*. **Gárriz & Miranda, 2020** studied the effects of heavy metal on sperm quality, fertilization and hatching rates, and embryo and larval survival of pejerrey fish (*Odontesthes bonariensis*).

Heavy metals pollution is a great concern for aquatic environments as they impart a wide range of toxicities with serious impacts to the aquatic faunal communities. From the present study it is suggested that precautionary measures should be taken against the discharge and treatment of this pollutant before releasing it in the fresh water bodies. This study calls for more attention to the protection and preservation of aquatic ecosystems, particularly those contaminated with heavy metals.

CONCLUSION:

In the present study, histopathological changes were observed in the Ovary of fish, *Channa gachua*, on exposure to heavy metal Nickel as (NiSO₄) at acute exposures. These interactions lead to reproductive failure in fishes. These alterations indicate that heavy metals may have direct or indirect effect on gonads. Hence a scientific method of detoxification is required to improve the health of these economic fishes.

REFERENCES:

1. **Ahmed, M. K., Kundu, G. K., Al-Mamun, M. H., Sarkar, S. K., Akter, M. S., and Khan, M. S. (2013).** Chromium (VI) induced acute toxicity and genotoxicity in freshwater stinging catfish, *Heteropneustes fossilis*. *Ecotoxicol. Environ. Saf.* 92, 64–70. doi:10.1016/j.ecoenv.2013.02.008
2. **Abdel-Warith, A. W. A., Younis, E. M., Al-Asgah, N. A., Abd-Elkader, M. O., and Elsayed, E. A. (2020b).** Effects of sub-lethal lead nitrate and copper sulfate concentrations on haematological parameters during long-term exposure in Nile tilapia (*Oreochromis niloticus*). *J. Sci. Ind. Res.* 79, 437–441.
3. **Bernet D., H. Schmidt, W. Meier, P. Burkhardt-Hol and T., Wahli, (1999):** Histopathology in fish: proposal for a protocol to assess aquatic pollution, *J. Fish Disease*, 22, 25-34.
4. **Bhat RA, Bakhshalizadeh S, Guerrera MC, Kesbiç OS, Fazio F., 2022:** Toxic effect of heavy metals on ovarian deformities, apoptotic changes, oxidative stress, and steroid hormones in rainbow trout. *J Trace Elem Med Biol.* 2023 Jan;75:127106. doi: 10.1016/j.jtemb.2022.127106. Epub 2022 Nov 11. PMID: 36402028.
5. **Bibak M, Sattari M, Tahmasebi S, Agharokh A, Imanpour Namin J., 2020:** Marine macro algae as a bio-indicator of heavy metal pollution in the marine environments, Persian Gulf. *Indian J Mar Sci.* 2020;49(3):357-63. <https://bit.ly/3utzlPQ>
6. **Dutta, H. M., and Dalal, R. (2008).** The effect of endosulfan on the ovary of bluegill sunfish: A histopathological study (*Lepomis macrochirus* sp). *Int. J. Environ. Res.* 2, 215–224.
7. **Gregory MR., 2009:** Environmental implications of plastic debris in marine settings: entanglement, ingestion, smothering, hangers-on, hitch-hiking and alien invasions. *Philos Trans R Soc Lond B Biol Sci.* 364:2013–2025. doi: 10.1098/rstb.2008.0265
8. **Ghazaly, K. S. (1992).** Sublethal effects of nickel on carbohydrate metabolism, blood and mineral contents of *Tilapia nilotica*. *Water Air Soil Pollut.* 64, 525–532. doi:10.1007/bf00483362
9. **Gárriz Á., Miranda L.A., 2020:** Effects of metals on sperm quality, fertilization and hatching rates, and embryo and larval survival of pejerrey fish (*Odontesthes bonariensis*) *Ecotoxicology.* 29:1072–1082.

10. **Gupta G., Srivastava P.P., Kumar M., Varghese T., Chanu T.I., Gupta S., Ande M.P., Jana P., 2021:** The modulation effects of dietary zinc on reproductive performance and gonadotropins' (FSH and LH) expression in threatened Asian catfish, *Clarias magur* (Hamilton, 1822) broodfish. *Aquac. Res.* 52:2254–2265.
11. **Gárriz Á., del Fresno P.S., Carriquiriborde P., Miranda L.A., 2019:** Effects of heavy metals identified in Chascomús shallow lake on the endocrine-reproductive axis of pejerrey fish (*Odontesthes bonariensis*) Gen. Comp. Endocrinol. 273:152–162.
12. **Han, J. M., Park, H. J., Kim, J. H., Jeong, D. S., and Kang, J. U. (2019).** Toxic effects of arsenic on growth, hematological parameters, and plasma components of starry flounder, *Platichthys stellatus*, at two water temperature conditions. *Fish. Aquat. Sci.* 22, 3–8. doi:10.1186/s41240-019-0116-5
13. **Hilmy, A. M., El-Domiaty, N. A., Daabees, A. Y., and Abdel-Latife, H. A. (1987).** Some physiological and biochemical indices of zinc toxicity in two freshwater fishes, *Clarias lazera* and *Tilapia zilli*. *Comp. Biochem. Physiology Part C Comp. Pharmacol.* 87, 297–301. doi:10.1016/0742-8413(87)90011-9
14. **Imtiaz Ahmed, Archo Zakiya, Francesco Fazio, 2022:** Effects of aquatic heavy metal intoxication on the level of hematocrit and hemoglobin in fishes: A review., *Front. Environ. Sci., Sec. Toxicology, Pollution and the Environment* Volume 10 - 2022 | <https://doi.org/10.3389/fenvs.2022.919204>
15. **Jamil Emon F, Rohani MF, Sumaiya N, Tuj Jannat MF, Akter Y, Shahjahan M, Abdul Kari Z, Tahiluddin AB, Goh KW., 2023:** Bioaccumulation and Bioremediation of Heavy Metals in Fishes-A Review. *Toxics.* 6;11(6):510. doi: 10.3390/toxics11060510. PMID: 37368610; PMCID: PMC10302055.
16. **Jurado, A.; Fernandes, M.; Videira, R.; Peixoto, F.; Vicente, J., 2011:** Herbicides: the face and the reverse of the coin. An in vitro approach to the toxicity of herbicides in non-target organisms. *Herbicides and environment.* 2011, 1-44, <https://doi.org/10.5772/12976>.
17. **Kevin V. Brix., Christian E. Schlekat., Emily R. Garman (2017):** The mechanisms of nickel toxicity in aquatic environments: An adverse outcome pathway analysis *Environ Toxicol and Chem*, Volume36, Issue5 2017, Pages 1128-1137
18. **Li Lin, Haoran Yang, Xiaocang Xu, 2022:** Effects of Water Pollution on Human Health and Disease Heterogeneity: A Review. *Front. Environ. Sci., Sec. Water and Wastewater Management*, Volume 10 – 2022 <https://doi.org/10.3389/fenvs.2022.880246>

19. **Lopes AR, Moraes JS, Martins CMG., 2022:** Effects of the herbicide glyphosate on fish from embryos to adults: a review addressing behavior patterns and mechanisms behind them. *Aquat Toxicol.* 251:106281. doi: 10.1016/j.aquatox.2022.106281. Epub 2022 Aug 27. PMID: 36103761.
20. **Patnaik, L and Patra, A.K., 2006:** Haematopoietic alterations induced by carbaryl in *Clarias batrachus* (LINN). *Journal of Applied Sciences and Environmental Management*, 10, 5-7, <https://doi.org/10.4314/jasem.v10i3.17305>.
21. **Martinez, C. B., Nagaie, M. Y., Zaia, C. T., and Zaia, D. A. (2004).** Acute morphological and physiological effects of lead in the neotropical fish *Prochilodus lineatus*. *Braz. J. Biol.* 64, 797–807. doi:10.1590/s1519-69842004000500009
22. **Shefali 1, Rahul Kumar 2, Mahipal Singh Sankhla 3, Rajeev Kumar 4 , Swaroop S. Sonone, 2021:** Review: Impact of Pesticide Toxicity in Aquatic Environment, *Biointerface Research In Applied chemistry*, Volume 11, Issue 3, 2021, 10131 – 10140., <https://doi.org/10.33263/BRIAC113.1013110140>
23. **E. Sherly Williams, V. Lekshmi Priya, L. Razeena Karim, 2022:** Bioaccumulation of heavy metals in edible tissue of crab (*Scylla serrata*) from an estuarine Ramsar site in Kerala, South India, *Watershed Ecology and the Environment*, Volume 4, Pages 59-65.
24. **Sattari M, Imanpour J, Bibak M, Forouhar Vajargah M, Khosravi A., 2019:** Investigation of metal element concentrations in tissue of *Rutilus frisii* in the Southwest Caspian Sea. *ISFJ.*; 28(3):149-161. <https://bit.ly/3FhZp5M>
25. **Shafir S, Rijn JV, Rinkevich B., 2007:** Short and long term toxicity of crude oil and oil dispersants to two representative coral species. *Environ Sci Technol.* 2007; 41:5571–5574. doi: 10.1021/es0704582
26. **Taslina K, Al-Emran M, Rahman MS, Hasan J, Ferdous Z, Rohani MF, Shahjahan M., 2022:** Impacts of heavy metals on early development, growth and reproduction of fish - A review. *Toxicol Rep.* 2022 Apr 20;9:858-868. doi: 10.1016/j.toxrep.2022.04.013. PMID: 36561955; PMCID: PMC9764183.

27. **Terence P Hughes¹, Maria J Rodrigues, David R Bellwood, Daniela Ceccarelli, Ove Hoegh-Guldberg, Laurence McCook, Natalie Moltschaniwskyj, Morgan S Pratchett, Robert S Steneck, Bette Willis, 2007:** Phase shifts, herbivory, and the resilience of coral reefs to climate change
Curr Biol. 2007 Feb 20;17(4):360-5. doi: 10.1016/j.cub.2006.12.049.
28. **Forouhar Vajargah M., Mohamadi Yalsuyi A., Sattari M., Prokić M.D., Faggio C., 2020:** Effects of copper oxide nanoparticles (CuO-NPs) on parturition time, survival rate and reproductive success of guppy fish, *Poecilia reticulata*. J. Clust. Sci. 31:499–506.
29. **Forouhar Vajargah M, Mohamadi Yalsuyi A, Hedayati A, Faggio C., 2018:** Histopathological lesions and toxicity in common carp (*Cyprinus carpio* L. 1758) induced by copper nanoparticles. Microscopy research and technique.;81(7):724-729. doi: 10.1002/jemt.23028
30. **Yalsuyi AM, Vajargah MF., 2017:** Acute toxicity of silver nanoparticles in Roach (*Rutilus rutilus*) and Goldfish (*Carassius auratus*). Journal of Environmental Treatment Techniques. 2017;5(1): 1-4. <https://bit.ly/3AY9i5Z>
31. **Yousif, R. A., Masyamsir, , Dhahiyat, , Sunarto, , and Zahidah, (2016).** Assessment the levels of heavy metals and water quality in Cikuda River, Indonesia. *Glob. J. Bio-Sci Biotechnol.* 5, 240–244.
32. **Yamaguchi S., Miura C., Ito A., Agusa T., Iwata H., Tanabe S., Tuyen B.C., Miura T., 2007:** Effects of lead, molybdenum, rubidium, arsenic and organochlorines on spermatogenesis in fish: monitoring at Mekong Delta area and in vitro experiment. *Aquat. Toxicol.* ;83:43–51
33. **Yan W., Hamid N., Deng S., Jia P.P., Pei D.S., 2020:** Individual and combined toxicogenetic effects of microplastics and heavy metals (Cd, Pb, and Zn) perturb gut microbiota homeostasis and gonadal development in marine medaka (*Oryzias melastigma*) J. Hazard. Mater. 397