AgriCos e-Newsletter
ISSN: 2582-7049
AgriCos e-Newsletter
Open Access Multidisciplinary Monthly Online Magazine
Volume: 06 Issue: 02 February 2025 Article No: 20

Assessment of Oxidative Stress Induced In Fish by Exposure to Heavy Metals

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SUMMARY

Heavy metal pollution in aquatic ecosystems has become a significant environmental concern due to the bioaccumulation and biomagnification of these contaminants through the food chain. Fish exposed to heavy metals experience oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) production and the antioxidant defense system. This study aims to evaluate oxidative stress induced in fish by exposing them to sublethal concentrations of heavy metals under controlled tank conditions for short-term (7 days) and long-term (21 days) durations. Blood serum samples collected post-exposure will be analysed for key oxidative stress markers, including Malondialdehyde (MDA), reduced Glutathione (GSH), Superoxide Dismutase (SOD), and Catalase (CAT), to assess oxidative damage and antioxidant defense levels. The findings are expected to provide valuable insights into the toxicological impacts of heavy metals on fish, enhancing understanding of oxidative stress responses. This research contributes to environmental risk assessment and the development of strategies to mitigate the harmful effects of heavy metal contamination in aquatic ecosystems.

INTRODUCTION

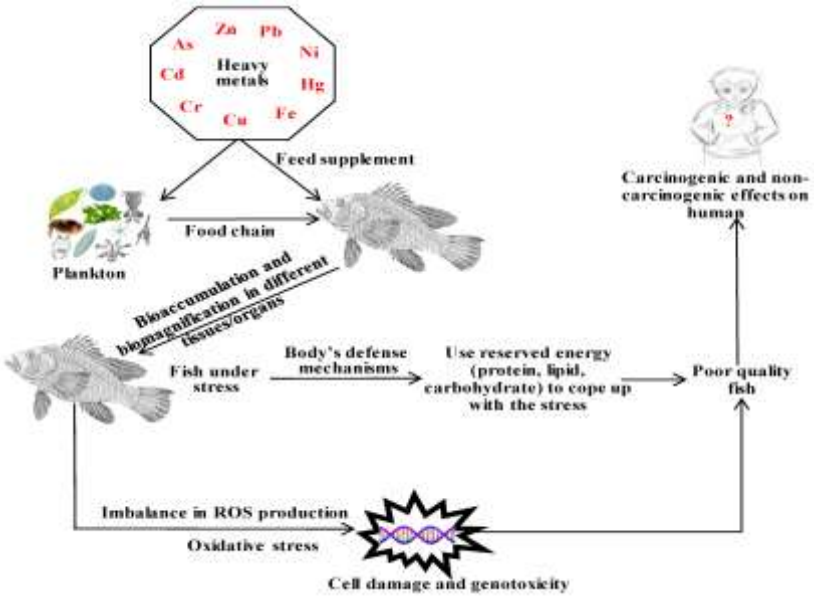
Oxidative stress occurs when there is an imbalance between reactive oxygen species (ROS) production and the antioxidant system's capacity to neutralize them. ROS, including free radicals like superoxide and hydroxyl radicals, are natural byproducts of cellular metabolism. While essential in small amounts for processes like cell signalling, excessive ROS can damage lipids, proteins, and DNA. Antioxidants, such as enzymes (e.g., catalase, superoxide dismutase) and molecules (e.g., vitamins C and E), help counteract ROS. However, when ROS levels surpass antioxidant defense, oxidative stress leads to cellular and organ dysfunction. This condition is associated with aging, diseases, and environmental stressors, including heavy metal pollution, making it a critical focus in health and environmental research.

Heavy metal contamination in aquatic ecosystems has become a critical environmental issue due to the enduring presence and steady accumulation of these toxic substances in nature. Fish can assimilate heavy metals through multiple pathways, such as consuming contaminated food, direct exposure to polluted water, absorption via the skin and gills, or ingestion of non-edible particles. These metals accumulate in various organs at different levels depending on the exposure route (Vinodhini and Narayanan, 2008). Once absorbed, heavy metals are transported through the bloodstream to various tissues, including the liver, where they undergo processes like modification, detoxification, and storage. Therefore, it is essential to analyse the levels of heavy metals in commercially significant fish species to evaluate potential health risks associated with their consumption (Cid *et al*., 2001). Fish are widely recognized as effective bioindicators for investigating the toxicological impacts of heavy metals in aquatic systems (Prabakaran *et al*., 2007). Enzymes, which are vital for regulating metabolic processes, can experience significant disruption in their normal activity due to heavy metal exposure. This disruption may lead to oxidative stress, a condition where essential organs sustain damage due to an imbalance between reactive oxygen species and the antioxidant defense system. Elevated levels of reactive oxygen species and a compromised antioxidant mechanism exacerbate this damage. Vital organs such as the liver and kidneys play a central role in maintaining the antioxidant defense in fish (Atli and Canli, 2008).

Oxidative Stress in Fish from Heavy Metals

- Heavy metals accumulate in fish through the food chain from industrial sources, leading to bioaccumulation and biomagnification. This results in oxidative stress in fish, caused by an imbalance in reactive oxygen species (ROS) production.

- When humans consume contaminated fish, it can lead to various health issues such as immune suppression, hypersensitivity to chemicals, breast cancer, reduced sperm count, and infertility. Therefore, conducting residue analysis of heavy metals in fish is crucial to assess and mitigate these health risks (Batool *et al.*, 2018).



Oxidative stress analysis

To evaluate oxidative stress caused by metal intoxication in fish, individuals will be exposed to varying sublethal concentrations of heavy metals (As, Zn, Pb, Cd, Cr, Cu, Fe, Hg, Ni) under controlled tank conditions for two different durations: 7 days (short-term exposure) and 21 days (long-term exposure). The exposure will take place in separate tanks for each metal, with the selected concentrations based on environmental relevance and previous research. After the specified exposure periods, blood serum will be collected to assess the effects of metals on oxidative stress. Fish will be anesthetized using an appropriate anaesthetic, and blood will be drawn from the caudal vein using a heparinized syringe to prevent clotting. The collected blood will then be centrifuged at 4000-10,000 rpm for 10 minutes at 4°C. The serum will be separated by decanting the clear supernatant, leaving the clot at the bottom. This supernatant will be preserved for subsequent enzyme analysis (Batool *et al.*, 2018). To analyse oxidative stress in the serum, the following oxidative stress markers and antioxidants will be measured to evaluate oxidative damage and antioxidant defense levels: Malondialdehyde (MDA), reduced Glutathione (GSH), Superoxide Dismutase (SOD), and Catalase (CAT).

Lipid peroxidation (MDA)

Lipid peroxidation in serum will be evaluated based on the reaction between MDA and thiobarbituric acid (TBA). Serum will be mixed with TBARS(thiobarbituric acid reactive substance reagent) which includes TCA (trichloroacetic acid) and TBA (thiobarbituric acid). The mixture will be heated in boiling water for 30 minutes. After cooling, the organic phase will be separated by centrifugation at 4000 rpm for 10 min. Supernatant will be collected and then the absorbance will be read at 530 nm using a spectrophotometer. Higher MDA levels indicate increased oxidative stress (Buege and Aust, 1978).

Superoxide dismutase (SOD U/L)

The activity of superoxide dismutase will be determined by measuring its ability to inhibit the photo reduction of nitro-blue tetrazolium (NBT) by superoxide. The enzyme's ability to neutralize superoxide will be measured by adding serum to a reaction mixture containing NBT (Nitro Blue Tetrazolium) and xanthine. The mixture will be incubated at 30°C for 10-15 minutes. After incubation, the inhibition of NBT reduction will be measured at 560 nm using a spectrophotometer. Higher SOD activity indicates stronger antioxidant defence (Giannopolitis and Ries, 1977).

Catalase (CAT)

The serum CAT activity will be determined using the method described by Aebi *et al*. (1974). First, H_2O_2 will be placed in the blank tube and phosphate buffer will be added to it. H_2O_2 will be placed in the sample tube and sample will be added to it and the tubes will be vortexed. The absorbance values will then be measured twice at 240 nm at 30 s intervals using the spectrophotometric method (Aebi *et al*., 1974).

Glutathione (GSH)

The GSH level will be determined using the method described by Tietz. First, serum will be diluted in phosphate buffer, and the first absorbance (OD_1) will be measured at 412 nm. Then Ellman's reagent will be added to the same tube, and the second absorbance value (OD_2) will be recorded at 412nm using a spectrophotometer (Tietz, 1969).

CONCLUSION

Exposure to heavy metals induces oxidative stress in fish by disrupting the balance between reactive oxygen species (ROS) production and the antioxidant defense system. The elevated ROS levels can lead to oxidative damage to vital biomolecules such as lipids, proteins, and DNA, impairing cellular functions and causing organ dysfunction. The analysis of oxidative stress markers, including Malondialdehyde (MDA), reduced Glutathione (GSH), Superoxide Dismutase (SOD), and Catalase (CAT), provides valuable insights into the extent of oxidative damage and the efficiency of the antioxidant response in fish. Understanding oxidative stress responses in fish exposed to heavy metals is essential for evaluating the toxicological impacts of pollutants in aquatic ecosystems. This knowledge helps identify species-specific vulnerabilities, inform environmental risk assessments, and develop strategies for mitigating the adverse effects of heavy metal contamination on aquatic life.

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