The Bioscan AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES 21(2): S.I (2), 2141-2148, 2026 www.thebioscan.com

ALTERATIONS IN TRACE ELEMENT CONCENTRATIONS ASSOCIATED WITH *PSEUDOMONAS AERUGINOSA* INFECTION IN *CYPRINUS CARPIO*

***Sunil Mohanrao Kadam* [1,2], *Sanjay Shamrao Nanware* [2*].**

1. *Department of Zoology, NES Science College, Nanded 431605, (M.S.), India.*
2. *Department of Zoology, Yeshwant Mahavidyalaya, Nanded 431602, (M.S.), India.*

** Corresponding author: Dr. Sanjay Shamrao Nanware*

E mail Id: drssnanware@gmail.com

DOI:https://doi.org/10.63001/tbs.2026.v21.i02.S.I(2).pp2141-2148

Keywords:

Cyprinus carpio, trace elements, Pseudomonas aeruginosa, mineral homeostasis, immune response, oxidative stress.

Received on:

20-05-2026

Accepted on:

08-06-2026

Published on:

18-06-2026

Abstract

Trace minerals are required for maintaining physiological function, immune response and metabolism in fish. This research examined the variations of trace elements in healthy and diseased fish to establish whether infection with *Pseudomonas aeruginosa* would affect mineral homeostasis. Boron (B), Manganese (Mn), Iron (Fe), Cobalt (Co), Copper (Cu), Zinc (Zn), Selenium (Se) and Molybdenum (Mo) concentrations were measured and compared among normal and infected fish.
Significant differences ($P < 0.05$) were found in concentrations of all trace elements studied. Infected fish had significantly elevated amounts of B, Mn, Fe, Co, Zn, Se, Mo than normal fish; whereas Cu was significantly decreased. Elevated trace elements could have been a result of increased metabolic rates; or an active immune response; or an increase in antioxidants during disease. Conversely, lower Cu levels suggest it is being used up at a faster rate in the physiological process of fighting against stress caused by pathogens.
In summary, this study has demonstrated that disease affects the balance of trace elements in fish; thereby affecting many physiological pathways which include health/disease resistance. Trace element analysis has shown promise to provide valuable indicators of fish health status and insight into the biochemical response of fish to infection.

Introduction

Common Carp (*Cyprinus carpio* Linnaeus, 1758) represents one of the most frequently cultured species of freshwater fish and, therefore, one of the major players in global aquaculture. Due to its fast growth rate and ability to thrive under a variety of environmental conditions along with the great market demand, common carp is considered as a primary species by many commercial fish producers (Horvath et al., 2023). While this is a problem, however, the use of intensive culture systems to grow common carp are exposing these fish to numerous types of stress, making them much more susceptible to disease caused by pathogens as it will have an adverse effect on both their growth rate and survival rate as well as affect their overall production (Senthamarai et al., 2023).

One of the bacterial pathogens responsible for infections among freshwater fish is *Pseudomonas aeruginosa*. It is known to be an opportunistic pathogen that can cause severe infections among those fish that are experiencing poor environmental conditions or are physiologically stressed (Baldissera et al., 2017). *P. aeruginosa* has been associated with infections that lead to hemorrhaging, ulcers, eroding fins, sepsis and death in a number of different species of fish. An infection caused by *P. aeruginosa* does not just damage tissue but also disrupts the normal physiological and biochemical processes occurring within the host organism (Thomas et al., 2014; Ghosh et al., 2024).

Trace minerals represent essential micronutrient elements necessary for performing a variety of biological functions including metabolic activity, activating enzymes, transporting oxygen, protecting against oxidative damage and regulating the immune system (Lall and Kaushik, 2021). These include iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), cobalt (Co), selenium (Se), and molybdenum (Mo), all of which are needed in very low quantities yet are critical to maintaining both good health and proper physiological function. Often times when an animal becomes infected the need for some of these trace minerals increases due to the fact that the host organism uses them to activate its defensive mechanisms to fight off invading pathogens and to restore damaged tissues (Chandrapalan and Kwong, 2021).

An infection caused by bacteria can result in significant changes in how trace elements are distributed throughout the body and how they are utilized. The changes that occur to how trace elements are distributed and used when a fish has been exposed to a pathogen, can be reflective of the degree of stress that the host fish is experiencing and will also aid in understanding the development of the disease process and the progress of the hosts' immune response (Hood and Skaar, 2012). Evaluating trace elemental concentrations in diseased animals will help researchers to develop a greater understanding of how organisms interact with their pathogens and make various biochemical alterations due to disease.

Due to the importance of trace elements to overall fish health; therefore, it was the goal of the present study to evaluate if there existed differences in the concentrations of 8 trace elements - boron

(B), manganese (Mn), iron (Fe), cobalt (Co), copper (Cu), zinc (Zn), selenium (Se), and molybdenum (Mo); in both healthy and Pseudomonas Aeruginosa-infected Cyprinus Carpio. The data collected from this investigation will assist investigators to identify how trace elements maintain homeostasis during an infection and potentially serve as indicators or biomarkers for evaluating the overall health status of fish.

Materials and methods:

Collection: Fish were collected from Barul dam, Nanded, Maharashtra.

Identification of fish: Fish samples were identified as per Day volume journal.

Identification of pathogen: The pathogen species has been identified by a 16S rRNA assay. The DNA was isolated and the 16S rRNA gene amplified by PCR. The amplified DNA fragments have then been aligned through the use of CLUSTALW within MEGA 11 software. These DNA fragments have then been manually trimmed and edited into full length 16S rRNA sequences. Confirmation of species is determined from a comparison of the two sequences using the percent sequence similarity score. A homology search was conducted against the NCBI Genbank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) utilizing the BLASTn program. An NJ tree was produced using MEGA 11 that contained all sites containing missing or gap information as well as utilized a total of 1000 bootstrap resampling iterations (Nei & Kumar, 2000; Tamura et al., 2021).

Mineral analysis:

Fish samples were oven dried and crushed into fine powder. Samples were prepared according to Alkaltham et al. (2020) to analyse iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), cobalt (Co), selenium (Se), boron (B) and molybdenum (Mo) using ICP-MS.

Results:

Trace element differences between healthy and infected fish were observed. Identification of *Pseudomonas aeruginosa* using 16s rRNA gene sequencing was also found.

Table 1. trace element data showing effect of *Pseudomonas aeruginosa* infection on trace elements. The boron (B) level rose from 15.19 + 0.19 ppm for healthy fish to 16.28 + 0.17 ppm for infected fish; $F = 56.13$; $p = .0017$. In addition, manganese (Mn) levels in infected fish were significantly greater (320.12 + 1.46 ppm), as opposed to those found in healthy fish (268.79 + 2.12 ppm); $F = 1175.45$; $p < .0001$. Iron (Fe) levels were likewise significantly greater in infected fish

(2062.97 + 11.58 ppm) than in healthy fish (1974.95 + 13.28 ppm); F = 73.78; p =.0010.Cobalt (Co) levels were significantly greater in infected fish (2.10 + 0.10 ppm) than in healthy fish (1.61 + 0.06 ppm); F = 54.98; p = .0018. Conversely, copper (Cu) levels dropped dramatically from 1123.74 + 7.75 ppm for healthy fish to 618.34 + 7.46 ppm for infected fish, and the decline was highly significant; F = 6632.36; p < .0001.

Finally, zinc (Zn) levels were significantly greater in infected fish (909.56 + 8.91 ppm) than in healthy fish (672.05 + 6.28 ppm); F = 1416.27; p < .0001.Additionally, selenium (Se) levels in infected fish (5.82 + 0.23 ppm) were significantly greater than those in healthy fish (3.46 + 0.17 ppm); F = 207.56; p = .0001. Similarly, molybdenum (Mo) was present at significantly higher levels in infected fish (6.84 + 0.26 ppm) than in healthy fish (4.40 + 0.20 ppm); F = 164.16; p = .0002.These findings demonstrate that infection results in significant changes in the homeostatic regulation of trace elements within fish tissue, resulting in an increase in B, Mn, Fe, Co, Zn, Se and Mo levels and a decrease in Cu levels.

Statistical analysis:

One-way analysis of variance (ANOVA) followed by Tukey test was performed by using instat 3 software for analysis of other data. All tests were performed in at least three replicates and the data are expressed as means ± standard deviation (SD). Significant differences compared to control are indicated by p value.

Discussion

Trace element content of the tissues of *C. carpio* were changed by the presence of *Pseudomonas aeruginosa*. It has long been understood that bacterial infections cause physiological stresses in organisms (Wendelaar Bonga, 1997), elicit immunological responses (Ellis, 2001) and hinder the normal functioning of metabolism (Magnadottir, 2010), all of which will likely impact the absorption, use and transport of trace minerals into different types of fish tissues.

Infected fish contained significantly larger amounts of trace elements than did healthy fish. Boron, Manganese, Iron, Cobalt, Zinc, Selenium and Molybdenum were all found at greater concentrations in infected fish. Increased levels of manganese and selenium could contribute to supporting antioxidant defense systems (Chandra & Roychoudhury, 2020).

During *P. aeruginosa* infection, reactive oxygen species are produced at higher rates increasing oxidative stress (da Cruz Nizer et al., 2021), thus, it is possible that the infected fish accumulated these trace elements to support the activity of antioxidant enzymes and to prevent damage to cells.

Increased levels of both iron and zinc were measured in infected fish. Both trace elements functionally participate in many aspects of immune response, cell metabolism and tissue repair (Hamad, 2025; Majumdar et al., 2025). It appears that the elevation observed may be due to the heightened metabolic needs of the host during the process of infection as well as the activation of the hosts defenses against the invading bacteria. Such changes in iron and zinc levels have previously been documented in fish exposed to pathogenic microbes and environmental stressors.

Elevated cobalt and molybdenum levels measured in infected fish may represent increased physiological activity directed toward maintaining homeostasis in response to bacterial infection (He et al., 2022). Additionally, a higher concentration of boron was found in infected fish possibly indicating borons role(s) in cellular/metabolic responses to stress/inflammation.

Copper concentrations were lower in infected fish than uninfected controls. Copper is required for numerous enzymes that are used for respiratory purposes or for antioxidant defense. Trace element profile analysis can also be used as a tool for assessing the physiological response of *Cyprinus carpio* exposed to *Pseudomonas aeruginosa* infection; and understanding the mechanisms underlying these responses. The alterations in trace elements were likely influenced by both the physiological adaptations required for survival due to the presence of the pathogen and/or by the activation of an immune response and subsequent production of reactive oxygen species associated with disease development. Therefore, trace element profiling is potentially a useful method for determining the health status of fish and how hosts respond to exposure to bacterial pathogens.

Conclusion

The current research indicated that *Pseudomonas aeruginosa* infection affected trace element content in the body of *Cyprinus carpio* (carp). Trace elements such as boron, manganese, iron, cobalt, zinc, selenium and molybdenum were found at higher concentration in infected than in non-infected carp. Concentration of copper was significantly lower in infected carp than in non-infected ones. This indicates an imbalance of trace element and activation of mechanisms associated with defence against infections, anti-oxidative stress protection and metabolic adjustments. It is possible to use the results on trace element analysis for the evaluation of the state of health of fish; therefore, it can also provide information about the response of fish hosts to infection by *P. aeruginosa.*

Acknowledgement: The authors are grateful to Principal, Yeshwant Mahavidyalaya, Nanded for providing laboratory facilities.

Authors contribution:

This work was carried out in collaboration among all authors. Author SN assisted with the creation of the manuscript and developed the entire project. Author SK authored a manuscript, conducted data analysis, and assisted with experimental activities. All authors read and approved the final manuscript.

Conflict of interest: Authors have declared that no competing interests exist.

References:

1. Horvath, L., Hegyi, A., Lefler, K. K., Csorbai, B., Kovacs, E., Szabo, T., ... & Urbanyi, B. (2023). Review of central-eastern European propagation and larvae nursing method for common carp (Cyprinus carpio L.). *Life*, *13*(12), 2334.
2. Senthamarai, M. D., Rajan, M. R., & Bharathi, P. V. (2023). Current risks of microbial infections in fish and their prevention methods: A review. *Microbial pathogenesis*, *185*, 106400.
3. Baldissera, M. D., Souza, C. F., Santos, R. C., da Rosa, L. V. C., Rosemberg, D. B., & Baldisserotto, B. (2017). Pseudomonas aeruginosa strain PAO1 infection impairs locomotor activity in experimentally infected Rhamdia quelen: Interplay between a stress response and brain neurotransmitters. *Aquaculture*, *47 3*, 74-79.
4. Thomas, J., Thanigaivel, S., Vijayakumar, S., Acharya, K., Shinge, D., Seelan, T. S. J., ... & Chandrasekaran, N. (2014). Pathogenecity of Pseudomonas aeruginosa in Oreochromis mossambicus and treatment using lime oil nanoemulsion. *Colloids and Surfaces B: Biointerfaces*, *116*, 372-377.
5. Ghosh, D., Mangar, P., Choudhury, A., Kumar, A., Saha, A., Basu, P., & Saha, D. (2024).

Characterization of a hemolytic and antibiotic-resistant Pseudomonas aeruginosa strain S3 pathogenic to fish isolated from Mahananda River in India. *PLoS One*, *19*(3), e0300134.

6. Lall, S. P., & Kaushik, S. J. (2021). *Nutrition and metabolism of minerals in fish. Animals 2021, 11, 2711*.
7. Chandrapalan, T., & Kwong, R. W. (2021). Functional significance and physiological regulation of essential trace metals in fish. *Journal of Experimental Biology*, *224*(24), jeb238790.
8. Hood, M. I., & Skaar, E. P. (2012). Nutritional immunity: transition metals at the pathogen–host interface. *Nature Reviews Microbiology*, *10*(8), 525-537.
9. Alkaltham, M.S., Salamatullah, A.M., Özcan, M.M., Uslu, N. and Hayat, K. (2020). The effects of germination and heating on bioactive properties, phenolic compounds and mineral contents of green gram seeds. LWT. 134: 110106.
10. Nei M. and Kumar S. (2000). Molecular Evolution and Phylogenetics. Oxford University Press, New York.
11. Tamura K., Stecher G., and Kumar S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution https://doi.org/10.1093/ molbev/msab120.
12. Wendelaar Bonga, S. E. (1997). The stress response in fish. *Physiological reviews*, *77*(3), 591-625.
13. Ellis, A. E. (2001). Innate host defense mechanisms of fish against viruses and bacteria. *Developmental & Comparative Immunology*, *25*(8-9), 827-839.
14. Magnadottir, B. (2010). Immunological control of fish diseases. *Marine biotechnology*, *12*(4), 361-379.
15. Chandra, S., & Roychoudhury, A. (2020). Role of selenium and manganese in mitigating oxidative damages. *Protective chemical agents in the amelioration of plant abiotic stress: Biochemical and Molecular Perspectives*, 597-621.
16. da Cruz Nizer, W. S., Inkovskiy, V., Versey, Z., Strempel, N., Cassol, E., & Overhage, J. (2021). Oxidative stress response in Pseudomonas

aeruginosa. *Pathogens*, *10*(9), 1187.

17. He, X. T., Li, X., Zhang, M., Tian, B. M., Sun, L. J., Bi, C. S., ... & Chen, F. M. (2022). Role of molybdenum in material immunomodulation and periodontal wound healing: Targeting immunometabolism and mitochondrial function for macrophage modulation. *Biomaterials*, *283*, 121439.
18. Hamad, M. (2025). The Fight for Iron: A Central Theme in Host-Pathogen Interactions. In *Metabolic Dynamics in Host-Microbe Interaction* (pp. 249-289). Singapore: Springer Nature Singapore.
19. Majumdar, A., Saraf, S. K., Sahu, C., Pathak, P., & Baghel, M. (2025). Zinc Deficiency: Impact on Growth, Immunity, and the Role of Plant-Based Nutritional Strategies. *Chemistry & Biodiversity*, *22*(10), e00325.
20. Bagheri, S., Gholamhosseini, A., Hoseinifar, S. H., & Banaee, M. (2024). Investigation of the effects of heavy metals (copper, cobalt, manganese, selenium, and zinc) on fish immune systems–an overview. *Annals of Animal Science*, *24*(4), 1025-1035.

Table 1. Effect of *Pseudomonas aeruginosa* infection on trace element composition.

Element	Normal fish (Mean ± SD)	Infected fish (Mean ± SD)	F-value	P-value
B	15.19 ± 0.19	16.28 ± 0.17	56.13	0.0017
Mn	268.79 ± 2.12	320.12 ± 1.46	1175.45	<0.0001
Fe	1974.95 ± 13.28	2062.97 ± 11.58	73.78	0.0010
Co	1.61 ± 0.06	2.10 ± 0.10	54.98	0.0018
Cu	1123.74 ± 7.75	618.34 ± 7.46	6632.36	<0.0001
Zn	672.05 ± 6.28	909.56 ± 8.91	1416.27	<0.0001
Se	3.46 ± 0.17	5.82 ± 0.23	207.56	0.0001
Mo	4.40 ± 0.20	6.84 ± 0.26	164.16	0.0002