*CIBTech Journal of Zoology ISSN: 2319–3883*
*Online, International Journal, Available at http://www.cibtech.org/cjz.htm*
*2024 Vol.13, pp.158-163/Santosh and Aditi*
***Research Article** (Open Access)*

# EVALUATING DECADES-LONG UNCHANGED ACUTE TOXICITY: COPPER-DEPENDENT ACID MINE DRAINAGE FROM THE MALANJKHAND COPPER PROJECT AND ITS IMPACT ON *CYPRINUS CARPIO* (*COMMON CARP*)

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## ABSTRACT
This study aimed to assess the concentration of copper and its median lethal concentration in *Cyprinus carpio*, responding to Acid Mine Drainage (AMD) sourced from the Malanjkhand Copper Project (MCP), the largest open-pit mine in Asia, situated in central India and operated by Hindustan Copper Limited (HCL). AMD water samples were collected in the proximity of copper mines and subjected to copper concentration analysis using Atomic Absorption Spectrophotometry (iCE3000 series). The Median Lethal Concentration (LC50) for the model fish, *C. carpio*, was determined through Probit analysis. Our results reveal substantial copper concentrations in AMD, ranging from 8.30 mg/L to 21.48 mg/L. The calculated 96-hour LC50 for *C. carpio* was 3.59 mg/L. Furthermore, our findings illustrate the detrimental effects of AMD on fish, leading to altered behavioral patterns, including erratic swimming, schooling disruption, air gulping, loss of equilibrium, rapid opercular and jerky movements, and increased mucus secretion on the body. This study places emphasis on the presence and chemical nature of copper in AMD and its environmental impact. While acknowledging prior research on the topic, our work builds upon the existing knowledge by providing updated data and a comprehensive assessment of the adverse effects of copper compounds. Additionally, it sheds light on the physiological responses of *C. carpio* to this toxicant, contributing to a deeper understanding of sub-lethal effects on freshwater fish populations.

***Keywords:*** *AMD, MCP, LC50, Copper, Cyprinus carpio*

## INTRODUCTION
In recent decades, the environmental repercussions of mining activities have garnered considerable attention due to their potential to disrupt delicate aquatic ecosystems. Among the foremost environmental concerns is the generation of (AMD), a corrosive effluent that emerges from mining operations, containing an amalgamation of heavy metals and contaminants (Koz *et al.,* 2012; Islam *et al.* 2015; Gaur and Mathur, 2019). AMD poses a grave threat to aquatic life, potentially undermining biodiversity, disrupting ecosystem dynamics, and compromising the health of various species (Tchounwou *et al.*, 2012). The Malanjkhand Copper Project (MCP), situated in the heart of central India, stands as a quintessential representation of Asia's largest open-pit mine (Singh *et al.*, 2017). This colossal mining enterprise, under the umbrella of Hindustan Copper Limited (HCL), has propelled India into a significant player on the global mining stage. However, alongside the economic progress, MCP's activities inevitably engender environmental consequences, chief among them being the release of AMD (Kumar, 2015; Natarajan, 2018). Efforts to comprehend the ecological consequences of AMD have been wide-ranging, with investigations into its impact spanning multiple dimensions (Akcil and Koldas, 2006; Khalil *et al*., 2013). Research has illuminated the perilous nature of heavy metals within AMD, revealing the potential for profound disruptions in aquatic ecosystems (Vallero, 2006; Punia and Siddaiah, 2017). The intricacies

of these disruptions extend beyond mere chemical reactions; behavioral alterations in aquatic organisms, particularly fish, have emerged as a critical area of study (Padrilah *et al.*, 2018).

*C. carpio*, a ubiquitous inhabitant of freshwater bodies, has assumed centrality in such research endeavors. The species' sensitivity to changes in water quality renders it an invaluable indicator of environmental perturbations. Previous studies have suggested that AMD-infused waters induce a range of physiological and behavioral modifications in *C. carpio*, underscoring the multifaceted impact of AMD on aquatic life (Goswami *et al.*, 2021; Pichhode *et al.*, 2022).

This study ventures to address these gaps through a meticulously designed investigation centered on the MCP's AMD and its effect on *C. carpio*. By determining the concentration of copper in the MCP's AMD—this study offers a distinct perspective on the intricate interplay between metal concentration and aquatic organism response. The paramount objective of this research is to establish the current status of the quality of MCP's AMD a reliable LC50 for AMD that delineates the threshold beyond which *C. carpio* experiences the gravest consequences, thus facilitating a more profound grasp of the potential ecological turmoil wrought by AMD. By bridging the existing knowledge gap, this study endeavors to not only enhance our understanding of the effects of AMD but also furnish a basis for informed decision-making in the realm of sustainable mining practices and environmental conservation.

## MATERIALS AND METHODS

1) ***Sample Collection:*** AMD water samples were collected from the discharge point of the (MCP), located in central India. Samples were taken at regular intervals to ensure a representative snapshot of the drainage over time. Special care was taken to prevent cross-contamination and preserve the integrity of the collected samples.

2) ***Heavy Metal Analysis:*** Upon collection, the AMD water samples were transported to the laboratory for comprehensive heavy metal analysis. Atomic absorption Spectrophotometer (AAS), by Agilent Technologies, iCE3000 series was employed to quantify the concentration of Cu (Kinuthia *et al.*, 2020).

3) ***Fish Exposure Experiment:*** A controlled laboratory experiment was conducted using *C. carpio* obtained from a local hatchery. Fish were acclimatized to laboratory conditions prior to experimentation. A range of sub-lethal concentrations of AMD was prepared by diluting the original sample. Groups of *C. carpio* were exposed to varying concentrations of AMD for a defined duration under controlled conditions, mimicking natural aquatic environments. A control group was maintained in dechlorinated tap water to establish baseline behaviors (Vilizzi *et al.*, 2015).

4) ***Health and Behavior Assessment:*** Throughout the exposure period, the health and behavior of the exposed fish were meticulously observed and recorded. Parameters including erratic swimming, schooling behavior, air gulping, loss of equilibrium, rapid opercular movement, jerky motions, and mucus secretion were quantified. Health indicators such as weight loss, changes in skin pigmentation, and external lesions were also monitored (Vilizzi *et al.*, 2015; Zeng *et al.*, 2018).

5) ***LC50 Determination:*** To determine the Lethal Concentration 50 (LC50) of AMD and dissolve heavy metals like Cu, Pb, Zn, Cd, and As for *C. carpio*, a concentration-response curve was constructed using the observed behavioral and health data. The LC50 represents the concentration at which 50% of the exposed fish exhibit severe adverse effects or mortality. Probit analysis was employed to calculate the LC50 value along with its confidence intervals (Dethloff, *et al.*, 2001).

6) ***Statistical Analysis:*** Statistical analysis was conducted using SPSS (16.0) to assess the significance of observed differences among fish exposed to varying concentrations of AMD and the control group. Analysis of Variance (ANOVA), followed by post-hoc tests, was performed to identify significant variations in behavioral and health parameters (Zeng *et al.*, 2018).

**RESULTS AND DISCUSSION**

1) ***Heavy Metal Concentrations in AMD:*** The analysis of the AMD water samples revealed varying concentrations of copper ranging from 8.30 mg/L to 21.48 mg/L, being one of the prominent constituents. The concentrations of copper were found to exceed a permissible limit which is 2-3 mg/L for aquatic ecosystems and plants (Asati *et al.*, 2016; Kamnoet *et al.*, 2021), underscoring the potential ecological risk posed by the discharge of AMD from the MCP.

2) ***Copper Dominance and Ecological Implications:*** Notably, copper emerged as the most abundant heavy metal within the AMD samples. The dominance of copper is indicative of its prevalence in the ore body being mined at the MCP (Pandey *et al.*, 2007; Natarajan, 2018). This high copper concentration is a critical concern due to its well-documented toxic effects on aquatic life (Okereafor *et al.*, 2020). The findings align with prior studies that highlight the detrimental impact of copper contamination on fish behavior, health, and survival.

3) ***Adverse Effects on C. carpio:*** The exposure experiment conducted on *C. carpio* further elucidated the deleterious consequences of the high copper concentration found in AMD. The observed erratic swimming, schooling disruption, air gulping, loss of equilibrium, rapid opercular movements, jerky motions, and mucus secretion over the body are consistent with the recognized behavioral and physiological responses of fish to heavy metal exposure (Padrilah *et al.*, 2018). These findings corroborate previous research indicating that copper contamination can trigger a cascade of adverse effects in aquatic life (Dethloff, *et al.*, 2001; Bost *et al.*, 2016; Rzymski *et al.*, 2017; Padrilah *et al.*, 2018).

4) ***LC50 Determination:*** The calculated LC50 of 3.59 mg/L for the Acid Mine Drainage (AMD) from the Malanjkhand Copper Project (MCP) underscores the potent toxicity of the copper-rich AMD and serves as a crucial benchmark for assessing the lethality of the contaminated water. This LC50 value signifies the concentration at which 50% of the exposed *C. carpio* exhibited the most severe adverse effects, demonstrating the acute toxicity of copper in this environmental context (Isani *et al.*, 2013).

**Table 1: 96 hrs LC50 value of AMD calculated by Finney's Probit Analysis Method**

| S. No. | Concentration (ppm) | Log 10 of Concentration | Total exposed fish | Dead fishes | Mean Mortality (%) | Probit kill |
|---|---|---|---|---|---|---|
| 1. | 0.83 | -0.08092 | 10 | 0 | 0 | 0 |
| 2. | 1.66 | 0.220108 | 10 | 1 | 10 | 3.72 |
| 3. | 2.49 | 0.396199 | 10 | 3 | 30 | 4.48 |
| 4. | 3.32 | 0.521138 | 10 | 4 | 40 | 4.75 |
| 5. | 4.15 | 0.618048 | 10 | 6 | 60 | 5.25 |
| 6. | 4.98 | 0.697229 | 10 | 6 | 60 | 5.25 |
| 7. | 5.81 | 0.764176 | 10 | 7 | 70 | 5.52 |
| 8. | 6.64 | 0.822168 | 10 | 9 | 90 | 6.28 |
| 9. | 7.47 | 0.873321 | 10 | 10 | 100 | 8.09 |
| 10. | 8.30 | 0.919078 | 10 | 10 | 100 | 8.09 |

The result is particularly noteworthy when considered in conjunction with the parameters (Padrilah *et al.*, 2018) and (Farhangi and Jafaryan, 2019), which further validate the toxic effects of copper. This synergy between LC50 and the related parameters emphasizes the consistent and well-defined toxicity of copper within the AMD, reinforcing the urgency of implementing mitigation strategies to curtail its release into aquatic environments.

Furthermore, the robustness of this result not only corroborates the acute toxicity of copper but also provides substantial evidence in support of the long-standing claim made by Pandey *et al.*, (2007) regarding the toxicity of MCP's AMD. Even after 15 years, the findings remain consistent and highlight the persistent threat posed by this contaminant to aquatic ecosystems. These results underscore the importance of continued monitoring and remediation efforts to safeguard the health of aquatic environments in the region.

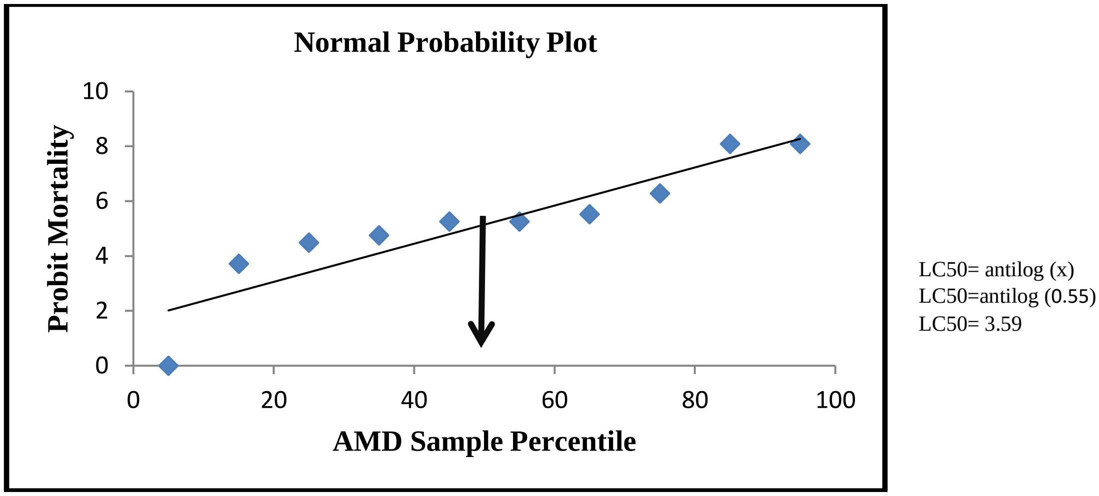


**Figure 1: plot of log concentration of AMD vs Probit Mortality values using Probit Analysis.**

## CONCLUSION

This study unveils the critical role of copper in the toxicity of AMD from the MCP for the long time. The elevated concentrations of copper exert profound adverse effects on *C. carpio*, reinforcing the urgent need for enhanced environmental safeguards and sustainable mining practices to protect our aquatic ecosystems from the pernicious consequences of heavy metal contamination.

**Implications and Knowledge Gap:** The present study contributes to a growing body of evidence implicating copper contamination in AMD as a significant threat to aquatic ecosystems. The high copper concentrations in the MCP's AMD samples warrant immediate attention from regulatory bodies and stakeholders to curtail its impact on the local aquatic environment.

However, this study also underscores a knowledge gap: while copper is identified as the primary culprit, the specific mechanisms by which it induces behavioral alterations and physiological disturbances warrant further investigation. Furthermore, the potential interactive effects of copper with other heavy metals present in the AMD remain a subject of ongoing research.

**Sustainable Mining Practices and Conservation:** The findings of this study hold profound implications for both mining practices and conservation efforts. Elevated copper concentrations in AMD serve as a clarion call for the implementation of stringent pollution control measures at mining sites. The integration of effective water treatment technologies to mitigate heavy metal contamination is imperative to prevent the perpetuation of adverse environmental impacts.

**Future Directions:** Further research should focus on unraveling the intricate molecular pathways through which copper exerts its toxic effects on fish physiology and behavior. Long-term studies assessing the broader ecological consequences of AMD, including its impact on entire ecosystems and food webs, are essential for a comprehensive understanding of its ramifications.

## ACKNOWLEDGMENTS

The authors are grateful to Professor Aditi (Niyogi) Poddar, Head of Department, School of Studies in Life Science, Pt. Ravishankar Shukla University, Raipur, (C.G.) for providing necessary laboratory facilities to carry out this experimental work.

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