INTERNATIONAL JOURNAL OF BIOSCIENCE FRONTIERS (IJBF)

ISSN: XXXX-XXXX
Page No.: 23-29
Doi:
Volume 1, Issue 1, July-September 2026

Dietary Moringa oleifera Attenuates Cadmium and Copper-Induced Haematological Toxicity and Restores Immune Homeostasis in the Catfish Clarias batrachus

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ABSTRACT

Heavy metal contamination of freshwater ecosystems by cadmium (Cd) and copper (Cu) is a major threat to fish health and aquaculture productivity, particularly across the industrially impacted river systems of the Indo-Gangetic plain. The present study evaluated the restorative and protective efficacy of dietary Moringa oleifera leaf meal against sublethal cadmium sulphate and copper sulphate toxicity in the air-breathing catfish, Clarias batrachus, with an emphasis on haematological derangement and immune homeostasis. Ninety-six-hour LC50 values were established at 12.42 mg/L (cadmium) and 0.58 mg/L (copper), and fish were chronically exposed to one-tenth LC50 concentrations for 60 days while receiving either a basal diet or a diet supplemented with 40% M. oleifera leaf inclusion (MLI). Both metals produced progressive anaemia, characterised by reduced total erythrocyte count (TEC), haemoglobin (Hb) and packed cell volume (PCV), together with leucocytosis, neutrophilia and lymphopenia indicative of a chronic inflammatory, immunosuppressive response. Dietary Moringa supplementation restored TEC by 76.3-78.6%, Hb by 71.4-73.7% and PCV by 68.2-68.3% of control values, while normalising the lymphocyte-neutrophil ratio and reducing erythrocyte morphological abnormalities by over 61%. In vitro batch-sorption assays confirmed that M. oleifera leaf powder chelates cadmium and copper (81.2% and 77.5% removal efficiency, respectively) through carboxylic acid and hydroxyl functional groups, a finding that explained 86.4% of the variance in in vivo protective efficacy. These results establish M. oleifera as a locally available, cost-effective dietary intervention capable of restoring haematological and immune homeostasis in metal-stressed catfish, with direct relevance to sustainable aquaculture in metal-contaminated tropical freshwater systems.

KEYWORDS
Clarias batrachus; Moringa oleifera; cadmium; copper; haematotoxicity; immunomodulation; aquaculture

ARTICLE HISTORY
Received: 15 July 2026
Accepted: 30 July 2026
Published: 14 August 2026

CITATION
Tyagi, N., Dietary Moringa oleifera Attenuates Cadmium and Copper-Induced Haematological Toxicity and Restores Immune Homeostasis in the Catfish Clarias batrachus. *International Journal of Bioscience Frontiers (IJBF),* 1(1), 23-29.
Doi

Introduction

Fish constitute an important, widely accessible source of dietary protein, and inland aquaculture makes a substantial contribution to food security across South Asia. However, both wild and cultured fish populations are increasingly exposed to heavy metal pollution arising from industrial effluents, agricultural run-off and urban wastewater discharge. Among the metals of greatest ecotoxicological concern, cadmium (Cd) and copper (Cu) are pervasive contaminants of Indian freshwater systems, including the Ganga and Yamuna basins (Stohs & Bagchi, 1995; Valko, Morris, & Cronin, 2005).

Cadmium is a non-essential, highly mobile metal that bioaccumulates in the liver, kidney and gills, generating reactive oxygen species (ROS) that provoke lipid peroxidation, protein denaturation and DNA fragmentation (Stohs & Bagchi, 1995). Copper, although an essential trace element at low concentrations, becomes acutely toxic at elevated waterborne levels, competitively inhibiting branchial Na^+/K^+-ATPase and Ca^{2+}-ATPase activity and precipitating ionoregulatory failure (Playle, Dixon, & Burnison, 1993). Both metals compromise erythropoiesis and immune competence, producing anaemia, leucocyte imbalance, and heightened susceptibility to opportunistic disease (Gill & Pant, 1987; Thophon *et al.*, 2003).

Moringa oleifera, the drumstick tree, is a fast-growing multipurpose plant whose leaves are exceptionally rich in vitamins, minerals, essential amino acids and antioxidant polyphenols, including quercetin, kaempferol and chlorogenic acid (Siddhuraju & Becker, 2003). These constituents confer antioxidant, anti-inflammatory, metal-chelating and immunomodulatory properties that have been documented across mammalian and finfish models (Makkar & Becker, 1996; Abdel-Latif, Abdel-Daim, Shukry, Nowosad, & Kucharczyk, 2022). Despite this evidence base, comprehensive investigations of *M. oleifera* efficacy specifically in *Clarias batrachus*, a commercially important, air-breathing catfish of the Indian subcontinent, remain scarce.

The present study was therefore designed to (i) characterise the acute and sublethal toxicity of cadmium sulphate and copper sulphate to *C. batrachus*, (ii) determine the haematological and immunological consequences of chronic sublethal exposure, and (iii) evaluate the restorative and protective efficacy of dietary *M. oleifera* leaf meal, integrating in vitro metal-chelation data with in vivo haematoprotection to establish a mechanistic basis for its use as a functional feed additive in metal-contaminated aquaculture systems.

2. Materials and Methods

2.1 Experimental Animals and Acclimatisation

Healthy, uniformly sized specimens of *Clarias batrachus* (80 ± 5 g) were procured, disinfected in 0.01% potassium permanganate, and acclimatised for two weeks in dechlorinated, aerated water maintained at 28 ± 2 degrees Celsius, pH 7.0-7.5, and dissolved oxygen above 5 mg/L, in accordance with APHA (2017) standard methods for water quality monitoring.

2.2 Acute Toxicity and Sublethal Concentration

Ninety-six-hour LC50 values were determined by probit analysis following a preliminary range-finding trial, yielding LC50 values of 12.42 mg/L for cadmium sulphate and 0.58 mg/L for copper sulphate. Sublethal concentrations for the chronic phase were fixed at one-tenth of the respective LC50 values (cadmium: 1.24 mg/L; copper: 0.058 mg/L), verified analytically by atomic absorption spectrophotometry.

2.3 Preparation of *Moringa oleifera* Leaf Meal and Experimental Diets

Fresh, authenticated *M. oleifera* leaves were shade-dried, ground into a fine powder, and incorporated into isonitrogenous, isocaloric pelleted diets at graded inclusion levels (0, 20, 40 and 60% MLI), following the basal formulation method of Eyo (1994). The 40% MLI diet, which produced optimal ameliorative effects without compromising palatability, was used for the principal restorative trial, consistent with reported inclusion ranges for omnivorous catfishes (Makkar & Becker, 1996).

2.4 Experimental Design and Sampling

Fish were allocated to five groups: an unexposed control; cadmium- and copper-exposed cohorts on the basal diet;

and parallel metal-exposed cohorts receiving the 40% MLI diet. Terminal sampling was performed at 30, 45 and 60 days post-exposure. Blood was drawn from the caudal haemal arch into K2EDTA vials for estimation of total erythrocyte count (TEC), total leucocyte count (TLC), haemoglobin (Hb, cyanmethemoglobin method), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), differential leucocyte counts (Leishman-stained smears) and erythrocyte morphology.

2.5 *In Vitro* Chelation Assay and Statistical Analysis

The metal-binding capacity of *M. oleifera* leaf powder was assessed by batch sorption assay, with residual metal quantified by atomic absorption spectrophotometry and functional group involvement confirmed by Fourier Transform Infrared (FTIR) spectroscopy. All haematological data were subjected to three-way ANOVA (toxicant x diet x duration) followed by Duncan's Multiple Range Test, with significance set at $p < 0.05$, using SPSS version 26.0.

3. Results

3.1 Acute Toxicity

Copper sulphate was approximately 21-fold more acutely toxic than cadmium sulphate to *C. batrachus* (LC50 0.58 vs. 12.42 mg/L), consistent with its rapid, direct branchial mode of action (Playle *et al.*, 1993), whereas cadmium's higher LC50 reflects its slower, cumulative systemic toxicokinetics.

3.2 Metal-Induced Haematological and Immune Derangement

Both metals produced progressive, time-dependent anaemia. By day 60, TEC and Hb had declined by 39.5% and 33.7% under cadmium exposure, and by 43.2% and 34.8% under copper exposure, relative to controls, with concurrent elevation of MCV and MCH indicating macrocytic, mildly hyperchromic anaemia. Erythrocyte morphological abnormalities, including poikilocytosis, anisocytosis and micronuclei, rose to 24.5% (cadmium) and 26.8% (copper) of the circulating erythrocyte population, reflecting oxidative membrane damage and genotoxic stress (Stohs & Bagchi, 1995).

Total leucocyte count rose by 41.6% (cadmium) and 45.9% (copper), driven by pronounced neutrophilia (12% in controls to 28-30% in exposed fish) and reciprocal lymphopenia (78% to 56-58%), shifting the lymphocyte-neutrophil ratio from approximately 6.5:1 to 2:1. This biphasic leucocytic pattern is characteristic of a chronic inflammatory, glucocorticoid-mediated immunosuppressive response to metal stress, and is consistent with the immunotoxic and haematopathic effects reported for other freshwater teleosts exposed to heavy metals (Gill & Pant, 1987).

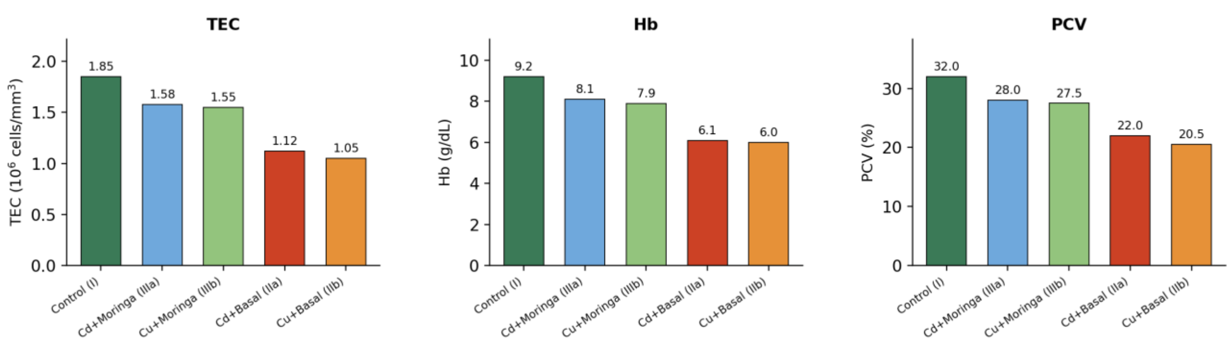
3.3 Moringa-Mediated Haematological and Immune Restoration

Dietary supplementation with 40% MLI markedly ameliorated these derangements. TEC was restored to 76.3% (cadmium) and 78.6% (copper) of control values by day 60, Hb to 71.4-73.7%, and PCV to 68.2-68.3%. Erythrocyte morphological abnormalities were reduced by 61.2-61.9%. Leucocyte profiles in supplemented groups were statistically indistinguishable from controls, with lymphocyte-neutrophil ratios restored to near-baseline levels, indicating substantive recovery of adaptive immune capacity. Duncan's Multiple Range Test confirmed that control and Moringa-supplemented groups formed statistically distinct, higher-ranking subsets relative to the metal-only basal-diet groups for every biomarker examined (Table 1).

Table 1: Haematological and histopathological biomarkers at day 60 across experimental groups, with Duncan's Multiple Range Test (DMRT) homogeneous subsets (n = 6 per group; values sharing a common letter within a row do not differ significantly, p > 0.05).

Parameter	Control (I)	Cd + Moringa (IIIa)	Cu + Moringa (IIIb)	Cd + Basal (IIa)	Cu + Basal (IIb)
TEC (10^6 cells/mm^3)	1.85 (a)	1.58 (b)	1.55 (b)	1.12 (c)	1.05 (c)
Hb (g/dL)	9.2 (a)	8.1 (b)	7.9 (b)	6.1 (c)	6.0 (c)
PCV (%)	32.0 (a)	28.0 (b)	27.5 (b)	22.0 (c)	20.5 (c)
TLC (cells/mm^3)	18,500 (a)	18,600 (a)	18,800 (a)	26,200 (b)	27,000 (b)
Liver HAI	1.5 (a)	5.8 (b)	6.5 (b)	16.2 (c)	17.6 (c)
Gill HAI	1.8 (a)	6.2 (b)	7.0 (b)	19.5 (c)	21.2 (c)
Kidney HAI	1.2 (a)	5.5 (b)	5.0 (b)	17.5 (c)	15.8 (c)

Figure 1. Haematological Recovery Conferred by Dietary Moringa oleifera Supplementation (40% MLI) at Day 60 Post-Exposure



3.4 *In Vitro* Chelation and Mechanistic Linkage

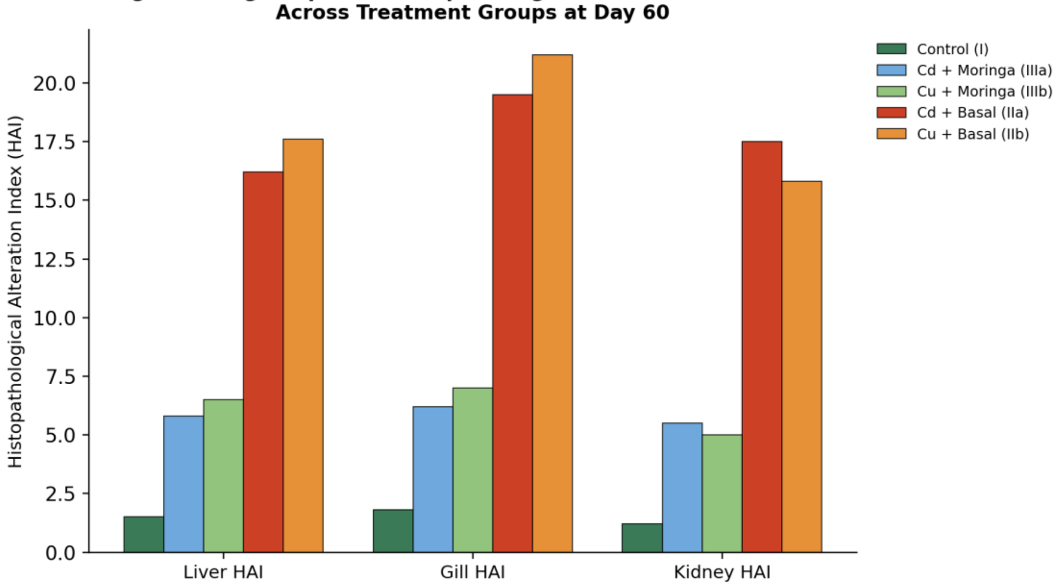
Batch sorption assays demonstrated equilibrium removal efficiencies of 81.2% for cadmium and 77.5% for copper by *M. oleifera* leaf powder, with FTIR spectroscopy implicating carboxylic acid and hydroxyl functional groups in metal binding. Linear regression indicated that in vitro removal efficiency explained 86.4% of the variance in in vivo protective efficacy ($p < 0.001$), directly linking the phytochemical chelating capacity of the leaf matrix to its organismal haematoprotective action. The organ- and endpoint-wise correspondence between chelation capacity and protective efficacy is summarised in Table 2, and the comparative severity of histopathological injury across organs and treatment groups is illustrated in Figure 2.

Table 2: Relationship between in vitro metal-chelating efficiency of *Moringa oleifera* leaf powder (batch sorption assay) and in vivo protective efficacy against cadmium- and copper-induced haematological and histopathological alterations at day 60 (standard deviations in parentheses)

Metal	*In vitro* removal (%)	Endpoint	Metal-only value	Moringa-supplemented value	*In vivo* protection (%)
Cadmium	81.2	TEC (10^6/mm^3)	1.12 (0.08)	1.58 (0.09)	76.3
		Hb (g/dL)	6.1 (0.4)	8.1 (0.4)	71.4
		PCV (%)	22.0 (1.5)	28.0 (1.5)	68.2
		Liver HAI	16.2 (1.5)	5.8 (1.0)	64.2
		Gill HAI	19.5 (1.6)	6.2 (1.1)	68.2
		Kidney HAI	17.5 (1.5)	5.5 (1.0)	68.6
Copper	77.5	TEC (10^6/mm^3)	1.05 (0.09)	1.55 (0.10)	78.6

		Hb (g/dL)	6.0 (0.5)	7.9 (0.5)	73.7
		PCV (%)	20.5 (1.4)	27.5 (1.6)	68.3
		Liver HAI	17.6 (1.6)	6.5 (1.1)	63.5
		Gill HAI	21.2 (1.8)	7.0 (1.2)	67
		Kidney HAI	15.8 (1.4)	5.0 (0.9)	68.4

Figure 2. Organ-Specific Histopathological Alteration Index (HAI) Across Treatment Groups at Day 60



4. Discussion

The findings confirm that both cadmium and copper induce clinically significant haematotoxicity and immune dysregulation in *C. batrachus*, operating through partially distinct but convergent mechanisms. Cadmium's slow, cumulative bioaccumulation in haematopoietic and detoxifying organs contrasts with copper's acute, surface-mediated disruption of branchial ion transport (Playle *et al.*, 1993), yet both metals converge on a common pathway of oxidative damage to erythrocyte membranes and haematopoietic tissue, mediated by reactive oxygen species and depletion of protein-bound sulfhydryl groups (Stohs & Bagchi, 1995; Valko *et al.*, 2005). The resultant anaemia, together with neutrophilia and lymphopenia, mirrors the classical stress-leucogram described for metal-exposed teleosts and signals an immunocompromised state that predisposes farmed fish to opportunistic disease even in the absence of overt mortality (Gill & Pant, 1987).

The comprehensive haematological and immunological restoration achieved through dietary *Moringa oleifera* supplementation is attributable to several convergent mechanisms. First, the leaf's rich content of bioavailable iron, folate and ascorbic acid directly supports erythropoiesis and haemoglobin synthesis (Makkar & Becker, 1996). Second, its polyphenolic constituents scavenge reactive oxygen species and stabilise erythrocyte membranes against oxidative haemolysis (Siddhuraju & Becker, 2003). Third, the demonstrated in vitro metal-chelating capacity, mediated by carboxylic acid and hydroxyl functional groups, reduces the bioavailability of free Cd2+ and Cu2+ ions for uptake by haematopoietic tissue, providing a direct physicochemical basis for the strong correlation observed between chelation efficiency and protective efficacy. Fourth, the immunomodulatory phytochemicals of *M. oleifera*, including polysaccharides and isothiocyanates, appear to counteract glucocorticoid-mediated lymphosuppression, restoring the lymphocyte-neutrophil balance that underpins adaptive immune competence (Abdel-Latif *et al.*, 2022).

These findings extend the growing evidence base for *M.*

oleifera as a functional aquafeed additive (Makkar & Becker, 1996; Abdel-Latif *et al.*, 2022) to a previously uncharacterised, ecologically and economically important air-breathing catfish species. Because *C. batrachus* tolerated the 40% inclusion level without evident feed refusal, the results suggest that this omnivorous species can accommodate higher Moringa inclusions than strictly herbivorous fish, an important practical consideration for cost-effective feed formulation in resource-limited tropical aquaculture. The strong statistical linkage between non-lethal haematological indices and the mechanistic chelation data further supports the use of peripheral blood parameters as sensitive, ethically preferable biomarkers for monitoring both metal exposure and therapeutic recovery in live fish.

Certain limitations should be acknowledged. The study was confined to organismal-level haematological endpoints and did not directly quantify tissue-level oxidative stress biomarkers or metal bioaccumulation, and a single Moringa inclusion level and exposure duration were examined. Future factorial studies incorporating graded metal and Moringa doses, extended exposure periods, and biochemical and molecular endpoints would further refine the therapeutic window and elucidate the precise signalling pathways, such as Nrf2-ARE activation, underlying Moringa-mediated cytoprotection.

5. Conclusion

Chronic sublethal exposure to cadmium and copper sulphate induces marked anaemia and immune dysregulation in *Clarias batrachus*, characterised by suppressed erythropoiesis, elevated erythrocyte abnormalities, and a neutrophilia-lymphopenia stress leucogram. Dietary supplementation with *Moringa oleifera* leaf meal at 40% inclusion substantially restores haematological parameters and normalises leucocyte differential profiles, an effect mechanistically linked to the leaf's demonstrated antioxidant, nutritional and metal-chelating properties. These findings position *M. oleifera* as a locally available, low-cost, and ecologically sustainable dietary intervention for mitigating heavy metal toxicity and preserving immune homeostasis in aquaculture systems affected by cadmium and copper contamination.

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