Phytoremediation Efficacy of Water Hyacinth (*Eichhornia crassipes*) in Heavy Metal Removal from Asa Dam: Toxicological Assessment in *Rattus norvegicus*

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Abstract- **Heavy metal contamination of freshwater bodies poses serious ecological and public health risks. Water hyacinth (*Eichhornia crassipes*) has emerged as a low-cost, eco-friendly biosorbent for heavy metal removal; however, the toxicological safety of water treated through this phytoremediation approach remains poorly characterized. This study evaluated the hematological and biochemical responses of *Rattus norvegicus* to Asa Dam water following water hyacinth biosorption treatment. Thirty-five adult rats (150–200 g) were randomly assigned to seven groups (n=5 per group): distilled water control, five graded concentrations of Asa Dam water (20%, 40%, 60%, 80%, and 100%), and a water hyacinth-treated dam water group. Animals received daily oral administration for 28 days. Complete blood counts, differential leukocyte counts, hepatic and renal function indices, serum proteins, bilirubin fractions, and electrolytes were assessed at study termination. Results: Erythrocyte parameters including packed cell volume (41.73–46.47%), hemoglobin (14.63–17.03 g/dl), red blood cell count, and erythrocyte indices (MCV, MCH, MCHC) remained unaltered across all groups ($P>0.05$). White blood cell counts declined significantly in groups receiving 40–100% dam water and water hyacinth-treated water ($P<0.05$). Monocyte percentages were significantly elevated in the 60%, 80%, and 100% groups. Serum AST, ALT, globulin, and urea showed notable increases, while creatinine and bilirubin fractions remained stable. Water hyacinth-mediated bioremediation of Asa Dam water demonstrated minimal hematotoxic effects in rats, with preservation of erythrocyte parameters and no anemic manifestations. The observed leukopenia and monocytosis warrant further investigation into immunological implications. These findings support the potential application of water hyacinth in phytoremediation systems while highlighting the necessity for comprehensive safety evaluations.**

Keywords: Biochemical, Heavy metals, Hematology, Water hyacinth

I. INTRODUCTION

Environmental pollution by heavy metals constitutes one of the most pressing global challenges, threatening both ecosystem integrity and human health (Hashem et al., 2023). Industrial effluents, agricultural runoff, and improper waste disposal have substantially elevated heavy metal concentrations in freshwater bodies, creating significant ecological and toxicological concerns (Rajaganapathy et al., 2024). Heavy metals including lead (Pb), cadmium (Cd), mercury (Hg), chromium (Cr), and arsenic (As) are particularly problematic due to their persistence, bioaccumulation potential, and adverse effects on biological systems even at trace concentrations (Briffa et al., 2020).

Conventional water treatment methods, including chemical precipitation, ion exchange, membrane filtration, and electrochemical techniques, often prove economically prohibitive and generate secondary pollutants requiring additional management (Burakov et al., 2018). Consequently, phytoremediation is the utilization of plants to remove, stabilize, or detoxify environmental contaminants has emerged as an eco-friendly and cost-effective alternative for heavy metal remediation (Ali et al., 2023; Pandey et al., 2024).

Water hyacinth, a free-floating aquatic macrophyte native to the Amazon basin, has garnered considerable attention for its exceptional heavy metal biosorption capacity (Shanab et al., 2022). Despite its classification as one of the world's most invasive aquatic species, water hyacinth exhibits remarkable growth rates (doubling biomass every 6-15 days under optimal conditions) and demonstrates extensive root systems with high surface area-to-volume ratios, facilitating efficient contaminant uptake (Rezania et al., 2023; Kumar et al., 2023). The plant's biosorption mechanisms involve multiple processes including ion exchange, surface complexation, electrostatic interactions, and precipitation on cellular structures (Mishra and Maiti, 2017; Aloo et al., 2023).

Previous investigations have documented water hyacinth's capacity to remove various heavy metals including Cr(VI), Pb(II), Cd(II), Cu(II), Ni(II), and Zn(II) from contaminated waters, with removal efficiencies often exceeding 80-95% under optimal conditions (Priya et al., 2021; Mahamadi, 2022). However, while the biosorption efficiency of water hyacinth has been extensively characterized, comprehensive toxicological evaluations of treated water remain limited. This knowledge gap is critical, as biosorption processes may alter water chemistry and potentially release phytotoxins or secondary metabolites that could pose health risks (Ndimele et al., 2021).

The Asa Dam, a significant water reservoir serving multiple purposes including irrigation, aquaculture, and domestic water supply in the region, faces persistent heavy metal contamination from anthropogenic activities (industrial discharge, agricultural practices, and urban runoff). Given the public health implications of utilizing dam water for various purposes, assessing the safety profile of water hyacinth-treated dam water is imperative.

Hematological and biochemical parameters serve as sensitive biomarkers for detecting systemic toxicity in laboratory animals, providing insights into the physiological status of various organ systems (Amadi et al., 2024). Alterations in complete blood counts, erythrocyte indices, and leukocyte differentials can indicate oxidative stress, hemolysis, immune dysfunction, or bone marrow suppression (Stockham and Scott, 2008; Adeyemi and Faniyan, 2023). Similarly, biochemical markers including hepatic enzymes (aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [ALP]), renal function indices (urea, creatinine), serum proteins, and electrolytes provide comprehensive assessment of hepatorenal integrity and metabolic homeostasis (Giannini et al., 2005; Olorunnisola et al., 2024).

This study aimed to evaluate the hematological and biochemical effects of Asa Dam water treated with water hyacinth biofilters using *Rattus* norvegicus as an experimental model. Specifically, we investigated: (1) hematological parameters including complete blood counts and differential counts; (2) biochemical markers of hepatic and renal function; and (3) serum electrolyte balance. The findings will contribute to understanding the safety profile of water hyacinth-mediated phytoremediation and inform decision-making regarding its practical application in water treatment systems.

II. MATERIALS AND METHODS

2.1 Study Location and Water Collection

Water samples were collected from Asa Dam, a multipurpose reservoir located in Kwara State, Nigeria. The dam serves agricultural, domestic, and industrial purposes and has documented heavy metal contamination from anthropogenic sources. Samples were collected in sterile polyethylene containers pre-cleaned with 10% nitric acid and rinsed with deionized water, following standard protocols (APHA, 2017). Collection occurred between 8:00-10:00 AM to minimize diurnal variations. Samples were immediately transported to the laboratory in ice-packed coolers and stored at 4°C until processing within 24 hours.

2.2 Water Hyacinth Collection and Preparation

Fresh water hyacinth (*Eichhornia crassipes*) plants were harvested from uncontaminated aquatic environments, authenticated by a botanist at the Department of Plant Biology, and voucher specimens deposited in the institutional herbarium. Plants were thoroughly washed with tap water to remove debris, followed by triple rinsing with distilled water. Root systems were separated, cut into approximately 2-3 cm segments, and used for biosorption experiments. The plant material was not subjected to drying or chemical pre-treatment to maintain natural biosorption properties.

2.3 Water Treatment Process

The biosorption process was conducted in batch mode using plastic containers. Fresh water hyacinth roots (200 g/l) were added to Asa Dam water and maintained under ambient conditions (temperature: 25±2°C, pH: 7.2±0.3) with gentle agitation for 72 hours to achieve equilibrium. The contact time and biosorbent dosage were optimized based on preliminary experiments. Following treatment, the water was filtered through Whatman No. 1 filter paper to remove plant material and particulates. The treated water was analyzed for physicochemical parameters and residual heavy metal concentrations using atomic absorption spectrophotometry (AAS) before animal administration.

2.4 Experimental Animals and Housing

Thirty-five healthy adult rats (*Rattus norvegicus*) weighing 150-200g were procured from the Animal House, Faculty of Veterinary Medicine. Animals were housed in polypropylene cages (5 rats per cage) under standard laboratory conditions: temperature 22±3°C, relative humidity 45-65%, and 12-hour light/dark photoperiod. Rats received standard pelleted rodent diet (crude protein ≥18%, crude fiber ≤5%, metabolizable energy ~3.0 kcal/g) and had ad libitum access to water. A 14-day acclimatization period preceded experimental procedures. All animal procedures complied with institutional ethical guidelines and the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

2.5 Experimental Design and Treatment Protocol

Animals were randomly assigned to seven groups (n=5 per group) using a randomization table: Group I (Control): Distilled water

Group II: 20% Asa Dam water + 80% distilled water

Group III: 40% Asa Dam water + 60% distilled water

Group IV: 60% Asa Dam water + 40% distilled water

Group V: 80% Asa Dam water + 20% distilled water

Group VI: 100% Asa Dam water

Group VII: Water hyacinth-treated Asa Dam water (100%).

Test solutions were administered orally via gavage at a volume of 10 ml/kg body weight daily for 28 consecutive days. Body weights were recorded weekly, and animals were monitored daily for clinical signs of toxicity including behavioral changes, food and water consumption, and mortality.

2.6 Sample Collection and Processing

Twenty-four hours after the final treatment, rats were fasted overnight (12 hours) with continued access to water. Animals were anesthetized using diethyl ether inhalation in a closed chamber. Blood samples (approximately 3-4 ml) were collected via cardiac puncture into two separate tubes: (1) EDTA-anticoagulated tubes for hematological analysis, and (2) plain tubes for biochemical analysis. Blood in EDTA tubes was gently mixed and analyzed within 2 hours of collection. Samples in plain tubes were allowed to clot at room temperature for 30 minutes, centrifuged at 3000 rpm for 15 minutes, and serum was carefully separated and stored at -20°C until biochemical analysis (within 48 hours).

2.7 Hematological Analysis

Complete blood counts were performed using an automated hematology analyzer (Sysmex KX-21N, Japan). Parameters evaluated included: Packed cell volume (PCV, %) using microhematocrit method; Hemoglobin concentration (Hb, g/dl) via cyanmethemoglobin method; Red blood cell count (RBC, $\times 10^{12}$/l); White blood cell count (WBC, $\times 10^{9}$/l); Platelet count ($\times 10^{9}$/l); and Erythrocyte indices: Mean corpuscular volume (MCV, fl), Mean corpuscular hemoglobin (MCH, pg), and Mean corpuscular hemoglobin concentration (MCHC, g/l). Differential leukocyte counts (neutrophils, lymphocytes, monocytes, eosinophils, basophils) were determined from Giemsa-stained blood smears under light microscopy at 1000× magnification, counting 100 cells per smear according to standard protocols (Jain, 1986).

2.8 Biochemical Analysis

Serum biochemical parameters were analyzed using a semi-automated biochemistry analyzer with commercially available diagnostic kits. Liver function tests included: Aspartate aminotransferase (AST, U/l) and Alanine aminotransferase (ALT, U/l) using the kinetic method (Reitman-Frankel method); Alkaline phosphatase (ALP, U/l) using p-nitrophenyl phosphate substrate; Total protein (g/l) and Albumin (g/l) using biuret and bromocresol green methods, respectively; Globulin (g/l) calculated as total protein minus albumin. Kidney function tests included: Urea (mmol/l) using urease-Berthelot method; and Creatinine (μmol/l) using Jaffe's kinetic alkaline picrate method. Bilirubin fractions (total, conjugated, and unconjugated) were determined using diazo method. Serum electrolytes (sodium, potassium, chloride, bicarbonate) were measured using ion-selective electrode methodology. All analyses were performed in duplicate, and the mean values were recorded.

2.9 Statistical Analysis

Data were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using SPSS software version 26.0. Normal distribution was assessed using Shapiro-Wilk test. Homogeneity of variance was evaluated using Levene's test. One-way analysis of variance (ANOVA) was employed to compare means across groups, followed by Tukey's post-hoc test for pairwise comparisons when significant differences were detected. Statistical significance was set at $P<0.05$. Graphs were generated using GraphPad Prism version 9.0.

III. RESULTS

3.1 General Observations and Mortality

Throughout the 28-day experimental period, no mortality or overt signs of toxicity were observed in any treatment group. Animals maintained normal feeding behavior, activity levels, and grooming patterns. No significant differences in body weight gain were recorded among groups ($P>0.05$), suggesting adequate nutritional status and absence of severe systemic toxicity.

3.2 Hematological Parameters

Comprehensive hematological parameters of rats following 28-day administration of Asa Dam water at various concentrations. Packed cell volume, hemoglobin concentration, red blood cell count, and erythrocyte indices showed no significant alterations across treatment groups. However, white blood cell counts demonstrated significant concentration-dependent reductions in groups receiving 40-100% dam water and water hyacinth-treated water. Monocyte counts significantly increased in 60%, 80%, and 100% treatment groups, while platelet counts showed variable responses as summarized in Table 1.

3.2.1 Erythrocyte Parameters

Packed cell volume (PCV) values ranged from 41.73±0.37% to 46.47±0.96% across all groups, with no statistically significant differences compared to control (43.17±1.2%) ($P>0.05$). Similarly, hemoglobin concentrations remained within normal physiological ranges (14.63±0.33 to 17.03±1.0 g/dl) across all treatment groups without significant alterations ($P>0.05$). Red blood cell counts showed no significant variations among groups, ranging from 7.54±0.14 to 8.91±0.42 $\times10^{12}$/l ($P>0.05$).

Erythrocyte indices including mean corpuscular volume (MCV: 51.00±0.5 to 55.93±3.02 fl), mean corpuscular hemoglobin (MCH: 18.60±0.46 to 19.97±0.37 pg), and mean corpuscular hemoglobin concentration (MCHC: 350±4.51 to 372.7±5.46 g/l) demonstrated no significant deviations from control values across all treatment concentrations ($P>0.05$), indicating preserved erythrocyte morphology and hemoglobin content.

3.2.2 Leukocyte Parameters

White blood cell counts exhibited concentration-dependent alterations. The 20% treatment group (18.60±1.6 $\times10^9$/l) showed no significant difference from control (20.53±0.16 $\times10^9$/l). However, groups receiving 40%, 60%, 80%, and 100% Asa Dam water, as well as the water hyacinth-treated group, demonstrated significant reductions ($P<0.05$): 14.03±0.61, 9.37±0.13, 10.43±0.367, 15.90±1.80, and 12.63±0.33 $\times10^9$/l, respectively. Notably, the 60% and 80% groups exhibited the most pronounced leukopenia.

Neutrophil percentages ranged from 9.60±1.10% to 26.10±4.37%, with the water hyacinth-treated group showing the highest values, though most groups remained statistically similar to control (11.30±0.55%, $P>0.05$). Lymphocyte percentages (70.30±5.80% to 82.80±0.46%) and monocyte percentages showed more pronounced variations. Monocyte counts significantly increased in 60% (7.80±0.2%), 80% (9.20±0.10%), and 100% (9.60±1.04%) treatment groups compared to control (5.90±0.61%, $P<0.05$).

3.2.3 Platelet Count

Platelet counts demonstrated variable responses across treatment groups. Control group platelet count was 665.3±5.67 $\times 10^9$/l. Significant elevations were observed in 60% (926.3±15.33 $\times 10^9$/l), 80% (921.0±23.00 $\times 10^9$/l), and water hyacinth-treated (832±18.67 $\times 10^9$/l) groups ($P<0.05$). Conversely, the 100% group showed significant reduction (548.3±34.67 $\times 10^9$/l, $P<0.05$).

Table 1: Hematological Parameters of Rats Following 28-Day Administration

Parameter	Control	20%	40%	60%	80%	100%	WH
PCV (%)	43.17±1.2	43.70±1.1^{a}	41.73±0.37^{a}	45.00±0.3^{a}	44.13±0.9^{a}	45.60±2.19^{a}	46.47±0.96^{a}
Hb (g/dl)	14.63±0.33	15.17±1.13^{a}	16.20±1.04^{a}	15.17±1.13^{a}	15.47±0.49^{a}	17.03±1.0^{a}	14.90±0.00^{a}
RBC ($\times 10^{12}$/l)	7.54±0.14	8.57±0.29^{a}	8.13±0.64^{a}	7.74±0.82^{a}	7.95±0.47^{a}	8.91±0.42^{a}	8.21±0.08^{a}
MCV (fl)	54.93±2.20	51.00±0.5^{a}	54.33±1.73^{a}	53.90±2.31^{a}	55.93±3.02^{a}	51.17±0.64^{a}	51.17±0.64^{a}
MCH (pg)	19.47±0.19	18.60±0.46^{a}	19.97±0.37^{a}	19.77±0.74^{a}	19.53±0.57^{a}	19.07±0.35^{a}	19.10±1.40^{a}
MCHC (g/l)	355±10.9	365±12.66^{a}	368±5.03^{a}	368±5.03^{a}	350±4.51^{a}	372.7±5.46^{a}	350.3±9.02^{a}
WBC ($\times 10^9$/l)	20.53±0.16	18.60±1.6^{a}	14.03±0.61^{b}	9.37±0.13^{bc}	10.43±0.37^{bc}	15.90±1.80^{bcd}	12.63±0.33^{b}
Neutrophil (%)	11.30±0.55	9.60±1.10^{ac}	16.57±0.96^{a}	10.10±1.1^{ac}	14.87±0.13^{ac}	13.87±1.07^{ac}	26.10±4.37^{b}
Lymphocyte (%)	82.80±0.46	76.53±7.9^{a}	71.53±3.7^{a}	79.87±1.24^{a}	70.30±5.80^{a}	72.87±4.22^{a}	79.33±2.29^{a}
Monocyte (%)	5.90±0.61	6.83±0.46^{a}	6.57±0.13^{a}	7.80±0.2^{b}	9.20±0.10^{b}	9.60±1.04^{b}	6.20±0.64^{a}
Platelet ($\times 10^9$/L)	665.3±5.67	699.3±37.33^{a}	645.7±16.33^{a}	926.3±15.33^{bcd}	921.0±23.00^{bcd}	548.3±34.67^{bcf}	832±18.67^{bcd}

Values presented as mean ± SEM (n=5). Superscript letters indicate statistical significance: ^{a} not significantly different from control; ^{b} significantly different from control; ^{c} significantly different from 20%; ^{d} significantly different from 40%; ^{f} significantly different from 60% ($P<0.05$). WH = Water Hyacinth-treated group.

3.3 Biochemical Parameters

Biochemical parameters following treatment administration. Aspartate aminotransferase (AST) levels showed significant elevation across all treatment groups. Serum globulin increased significantly in 20% and 40% groups. Total protein increased in 20% and 40% groups, while urea concentrations showed significant elevations across all treatment groups. Other parameters including creatinine, bilirubin fractions, and most electrolytes remained relatively stable with some groups showing mild alterations as discussed in Table 2.

3.3.1 Hepatic Function Markers

Aspartate aminotransferase (AST) levels showed elevation in certain treatment groups, though specific values were not uniformly significant across all concentrations. Alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities remained within normal reference ranges across most treatment groups, with some groups showing mild non-significant elevations compared to control (P>0.05). Total serum protein levels increased in the 20% and 40% treatment groups compared to control, while other groups showed no significant alterations. Serum albumin levels remained stable across all groups. Globulin concentrations demonstrated increases in specific treatment groups, with the 40% group showing the most pronounced elevation.

3.3.2 Renal Function Markers

Serum urea concentrations showed increases across various treatment groups. Creatinine levels remained within normal physiological ranges across all treatment groups, indicating preserved glomerular filtration capacity and absence of significant nephrotoxicity (P>0.05).

3.3.3 Bilirubin Metabolism

Total bilirubin, conjugated bilirubin, and unconjugated bilirubin levels showed no significant differences among all treatment groups compared to control (P>0.05), suggesting normal hepatic conjugation capacity and absence of cholestatic or hemolytic effects.

3.3.4 Serum Electrolytes

Sodium concentrations showed reductions in certain treatment groups. Chloride ion and bicarbonate levels demonstrated increases in some treatment groups compared to control. These electrolyte alterations suggest potential effects on acid-base balance and renal tubular function, though values generally remained within acceptable physiological ranges.

Table 2: Biochemical Parameters of Rats Following 28-Day Administration

Parameter	Control	20%	40%	60%	80%	100%	WH
Total protein (g/dl)	6.19±0.19	7.19±0.23^{a}	7.89±0.81^{bc}	6.60±0.18^{a}	6.72±0.23^{a}	6.43±0.19^{a}	7.36±0.23^{a}
Albumin (g/dl)	2.34±0.08	2.37±0.24^{a}	2.34±0.03^{a}	2.40±0.13^{a}	2.58±0.18^{a}	2.48±0.09^{a}	2.70±0.14^{a}
Globulin (g/dl)	3.70±0.26	4.82±0.28^{b}	6.11±0.52^{b}	4.12±0.17^{ac}	4.14±0.28^{ac}	3.95±0.29^{ac}	4.65±0.15^{ac}
ALP (U/l)	52.75±0.94	51.53±1.10^{a}	53.59±0.79^{a}	53.77±0.91^{a}	52.73±1.1^{a}	52.03±0.30^{a}	54.67±2.09^{a}
ALT (U/l)	102.70±5.52	122.1±4.45^{b}	133.4±5.3^{b}	123±5.7^{b}	118.4±5.02^{a}	140.1±4.6^{b}	129.5±5.19^{b}
AST (U/l)	27.11±1.15	41.25±2.9^{b}	42.73±1.7^{b}	40.44±2.28^{b}	35.29±1.02^{b}	41.57±2.16^{b}	42.41±1.04^{b}
Bicarbonate (mmol/l)	46.92±2.35	50.02±1.05^{a}	57.47±2.59^{a}	54.24±5.0^{a}	53.16±4.16^{a}	56.31±3.35^{a}	51.27±0.94^{a}
Chloride (mmol/l)	69.81±1.12	65.52±1.12^{b}	67.63±1.03^{acd}	65.58±0.25^{b}	66.38±0.43^{ac}	67.55±0.91^{a}	62.50±0.62^{bd}
Sodium (mmol/l)	163.9±6.67	143.5±2.7^{b}	143.9±0.98^{b}	144.7±3.8^{b}	144.6±2.07^{b}	145.1±2.4^{b}	147±2.54^{b}
Total bilirubin (µmol/l)	45.40±0.89	43.34±4.8^{a}	42.13±4.18^{a}	40.68±2.7^{a}	36.08±1.07^{a}	38.98±1.48^{a}	34.62±0.73^{a}
Creatinine (µmol/l)	18.36±1.30	18.36±1.30^{ac}	18.36±3.40^{ac}	18.36±1.30^{ac}	23.63±2.27^{ac}	11.80±0.00^{acd}	15.73±2.27^{ac}
Conjugated bilirubin (µmol/l)	9.67±0.05	8.93±0.05^{a}	9.82±0.4^{a}	8.98±0.35^{a}	9.33±0.17^{a}	9.48±0.17^{a}	10.46±0.48^{a}

Unconjugated bilirubin (μmol/l)	34.35±0.70	35.53±4.1^{ac}	32.34±3.14^{a}	32.07±2.9^{a}	27.80±0.19^{a}	30.12±0.52^{a}	28.27±0.84^{a}
Urea (mg/dl)	46.69±4.00	94.84±6.6^{b}	105.2±2.27^{b}	87.62±3.5^{b}	93.86±1.02^{b}	73.97±9.8^{bc}	102.4±2.14^{bc}

Values presented as mean ± SEM (n=5). Superscript letters indicate statistical significance: ^{a} not significantly different from control; ^{b} significantly different from control; ^{c} significantly different from 20%; ^{d} significantly different from 40% (P<0.05). WH = Water Hyacinth-treated group. ALP = Alkaline phosphatase; ALT = Alanine aminotransferase; AST = Aspartate aminotransferase.

IV. DISCUSSION

This investigation evaluated the hematological and biochemical safety profile of Asa Dam water treated with water hyacinth biofilters. The preservation of erythrocyte parameters indicates absence of significant hematotoxicity, aligning with findings by Addass et al. (2010). The concentration-dependent leukopenia observed warrants attention from an immunological perspective, as it may compromise host defense mechanisms (Kumar et al., 2022). The monocytosis in higher concentration groups may represent compensatory responses to environmental stress (Mitchell et al., 2002). Hepatic enzyme elevations indicate mild hepatocellular injury consistent with heavy metal exposure (Yang et al., 2014). Importantly, water hyacinth-treated water produced biological effects comparable to untreated water, suggesting that current biosorption protocols require optimization (Priya et al., 2021).

4.1 Erythrocyte Parameters and Anemia Assessment

The preservation of packed cell volume, hemoglobin concentration, red blood cell count, and erythrocyte indices across all treatment groups indicates the absence of significant hematotoxic effects on erythropoiesis. These findings align with previous research by Addass et al. (2010) and corroborate observations by Jahn (1988) regarding the minimal erythrotoxic potential of certain water treatment systems. The maintained PCV values (41.73-46.47%) fall within the normal reference range for rats (37-49%), suggesting that neither untreated nor water hyacinth-treated Asa Dam water induced hemolytic, hemorrhagic, or bone marrow suppressive effects (Delwatta et al., 2018; Amadi et al., 2024).

The stability of erythrocyte indices (MCV, MCH, MCHC) is particularly significant, as these parameters are sensitive indicators of iron metabolism, vitamin B12/folate status, and chronic heavy metal exposure (Adeyemi and Faniyan, 2023). Heavy metals, particularly lead and cadmium, are known to interfere with heme synthesis and cause microcytic or normocytic anemia through multiple mechanisms including inhibition of δ-aminolevulinic acid dehydratase (ALAD), disruption of iron homeostasis, and oxidative damage to erythrocyte membranes (Flora et al., 2012; Rehman et al., 2018). The absence of such alterations suggests either effective heavy metal removal by water hyacinth biosorption or concentrations below the threshold for erythrotoxicity.

4.2 Leukocyte Dynamics and Immunological Implications

The most striking hematological finding was the concentration-dependent reduction in white blood cell counts observed in groups receiving 40-100% Asa Dam water and water hyacinth-treated water. White blood cells constitute the primary cellular component of innate and adaptive immunity, and leukopenia can compromise host defense mechanisms against infectious agents (Olorunisola et al., 2012; Kumar et al., 2022). The reduction from control values (20.53×10^{9}/l) to as low as 9.37×10^{9}/l in the 60% group represents approximately a 54% decrease, which, while maintaining values within the lower reference range for rats ($6\text{-}17\times10^{9}$/l), warrants careful interpretation (Petterino and Argentino-Storino, 2006).

Several mechanisms may explain the observed leukopenia. First, residual heavy metals in treated water, despite biosorption, could exert immunosuppressive effects through oxidative stress induction, disruption of cellular signaling pathways, and direct cytotoxicity to hematopoietic progenitor cells (Hemdan et al., 2006; Bhattacharya, 2022).

Cadmium, for instance, can suppress lymphocyte proliferation and induce apoptosis in immune cells through mitochondrial dysfunction and caspase activation (Nair et al., 2013). Second, the biosorption process might release plant-derived compounds with immunomodulatory properties, though this hypothesis requires further investigation through phytochemical analysis of treated water.

Interestingly, the leukopenic effect was not strictly dose-dependent, as evidenced by the more pronounced reduction in 60% and 80% groups compared to the 100% group. This non-linear response could reflect hormetic effects, where intermediate concentrations trigger maximal biological responses, or might indicate complex interactions between multiple contaminants present in dam water (Calabrese and Baldwin, 2002; Zhou et al., 2023). The water hyacinth-treated group also exhibited leukopenia (12.63×10^9/l), suggesting that while biosorption may reduce heavy metal burden, it does not completely eliminate immunosuppressive potential, possibly due to incomplete metal removal or formation of novel compounds during the biosorption process.

4.3 Differential Leukocyte Counts and Cellular Immunity

The differential leukocyte analysis revealed preservation of neutrophil and lymphocyte percentages across most treatment groups, aligning with findings reported by Olorunisola et al. (2012). Lymphocytes, comprising 70-80% of total leukocytes in this study, play crucial roles in adaptive immunity, including antibody production, cell-mediated immunity, and immunological memory (Abbas et al., 2018). The maintenance of lymphocyte percentages suggests that the observed leukopenia affects leukocyte populations proportionally rather than selectively targeting specific lineages.

However, the significant increase in monocyte counts in 60%, 80%, and 100% treatment groups (7.80-9.60% vs. control 5.90%) merits attention. Monocytes and their tissue-resident derivatives (macrophages) are essential components of innate immunity, participating in phagocytosis, antigen presentation, and secretion of inflammatory mediators (Gordon and Taylor, 2005; Italiani and Boraschi, 2014). The monocytosis observed could represent a compensatory response to environmental stress or low-grade inflammation induced by waterborne contaminants. According to Mitchell et al. (2002), elevated monocyte counts can indicate enhanced phagocytic activity as an adaptive response to xenobiotic exposure. Alternatively, this finding might reflect reactive monocytosis secondary to tissue injury or inflammatory processes not yet manifested in other biochemical parameters (Ginhoux and Jung, 2014).

The water hyacinth-treated group exhibited elevated neutrophil percentages (26.10%) compared to control (11.30%), though this difference did not reach statistical significance in the complete analysis. Neutrophils constitute the first line of cellular defense against bacterial pathogens, and their elevation could suggest a subclinical inflammatory response or stress-induced granulocytosis (Afolayan and Yakubu, 2009). The decreased neutrophil counts observed by some researchers in similar studies may have consequential effects on immune system function and phagocytic activity (Afolayan and Yakubu, 2009), highlighting the complexity of immunological responses to environmental contaminants.

4.4 Platelet Function and Hemostatic Implications

Platelet count variations observed across treatment groups present an intriguing finding. The significant thrombocytosis in 60%, 80%, and water hyacinth-treated groups ($832\text{-}926\times10^9$/l) compared to control (665×10^9/l) contrasts with the thrombocytopenia observed in the 100% group (548×10^9/l). These findings partially align with observations by Gunatilake et al. (1996), who reported platelet alterations following toxic exposures in rats, though the mechanisms differ.

Reactive thrombocytosis can occur secondary to inflammatory states, iron deficiency, malignancy, or as a compensatory response to chronic low-grade bleeding (Schafer, 2004; Bleeker and Hogan, 2011). Heavy metals, particularly arsenic and lead, can affect megakaryopoiesis through oxidative stress mechanisms and disruption of thrombopoietin signaling (Hernández and Tsuchiya, 2018). The biphasic platelet response initially increase at intermediate concentrations followed by decrease at the highest concentration which might reflect dose-dependent shifts between compensatory thrombopoiesis and direct toxicity to megakaryocytes or peripheral platelet destruction.

The thrombocytopenia in the 100% group, while maintaining counts within acceptable ranges for rats (500-1300×10^9/l), could indicate increased platelet consumption through activation of coagulation pathways, direct platelet toxicity, or splenic sequestration (George, 2000; Kaushansky, 2005). These hemostatic alterations, though not severe, warrant monitoring in long-term exposure scenarios and highlight the need for comprehensive coagulation studies including prothrombin time, activated partial thromboplastin time, and platelet function assays.

4.5 Hepatic Function and Metabolic Homeostasis

The elevation of aspartate aminotransferase (AST) in certain treatment groups confirms previous reports by Aleman et al. (1998) and aligns with the paradigm of hepatocellular injury. AST and ALT are intracellular enzymes normally present at low concentrations in serum; their elevation typically indicates hepatocyte damage and subsequent enzyme leakage into systemic circulation (Yang et al., 2014; McGill, 2016). Heavy metals can induce hepatotoxicity through multiple mechanisms: oxidative stress generation via Fenton reactions, disruption of mitochondrial function, interference with calcium homeostasis, activation of apoptotic pathways, and direct binding to cellular macromolecules (Ercal et al., 2001; Jaishankar et al., 2014).

The preservation of alkaline phosphatase (ALP) within normal ranges is noteworthy, as ALP elevation typically indicates cholestasis or bone pathology (Sharma et al., 2014). The pattern of AST elevation without proportionate ALP increase suggests hepatocellular injury without significant biliary obstruction. This interpretation is further supported by the absence of bilirubin elevations, which would be expected in cholestatic injury (Giannini et al., 2005).

The increase in total serum protein and globulin fractions in certain treatment groups, as reported by Radostits et al. (2007) and Ahmed et al. (1992), could reflect several physiological responses. Globulins, including immunoglobulins and acute-phase proteins, typically increase during inflammatory states, infections, or chronic antigenic stimulation (Tothova et al., 2014). The elevated serum globulin might indicate a low-grade inflammatory response to waterborne contaminants or adaptive immune activation. Alternatively, dehydration or hemoconcentration could artifactually elevate protein concentrations, though the maintained hematocrit values argue against this explanation.

The increased serum protein in affected groups may indicate impairment in normal hepatic function, as the liver is the primary site of albumin synthesis and plays crucial roles in protein metabolism (Garba and Abubakar, 2007; Ahmed et al., 1992). However, the maintained albumin levels suggest preserved hepatic synthetic capacity, indicating that any hepatocellular injury detected through enzyme elevation has not progressed to hepatic insufficiency.

4.6 Renal Function and Nitrogenous Waste Metabolism

The elevation of serum urea without corresponding creatinine increases presents an interesting metabolic pattern. Urea, synthesized in the liver from ammonia (a product of amino acid catabolism), serves as the primary vehicle for nitrogen excretion (Banerjee, 2007). Elevated urea can result from increased protein catabolism, gastrointestinal bleeding, dehydration, or impaired renal excretion (Schrier, 2010). The preserved creatinine levels, consistent with findings by Ojo et al. (2014), suggest maintained glomerular filtration rate, as creatinine is more specifically indicative of renal function and less affected by dietary protein or metabolic factors (Stevens and Levey, 2009).

This dissociation between urea and creatinine could indicate enhanced protein catabolism secondary to metabolic stress, mild dehydration, or increased hepatic urea synthesis without significant nephron damage (Schrier, 2010). Heavy metals can induce cellular stress responses that promote protein degradation through ubiquitin-proteasome and autophagy-lysosome pathways (Dong et al., 2015; Wang and Korolchuk, 2022). Additionally, the liver's central role in detoxification might lead to increased metabolic activity and consequent elevation of urea production.

The absence of creatinine elevation is particularly reassuring, as this indicates preservation of glomerular filtration capacity and absence of significant tubular dysfunction. Heavy metals, particularly cadmium, mercury, and lead, are well-established nephrotoxins capable of inducing tubular necrosis, glomerular sclerosis, and interstitial fibrosis (Barbier et al., 2005; Järup, 2003). The maintained creatinine levels suggest that either heavy metal concentrations in the water were below nephrotoxic thresholds or that water hyacinth biosorption effectively reduced the renal toxic burden.

4.7 Bilirubin Metabolism and Hepatobiliary Function

The absence of alterations in total, conjugated, and unconjugated bilirubin levels across all treatment groups provides strong evidence for preserved hepatobiliary function and absence of hemolytic processes. Bilirubin, derived from heme catabolism, undergoes hepatic conjugation before biliary excretion (Vítek and Tiribelli, 2021). Unconjugated (indirect) bilirubin elevation typically indicates increased erythrocyte destruction or impaired hepatic uptake/conjugation, while conjugated (direct) bilirubin elevation suggests hepatocellular injury or biliary obstruction (Fevery, 2008).

The maintained bilirubin levels corroborate the erythrocyte findings, further confirming the absence of hemolytic anemia. Additionally, normal conjugated bilirubin indicates preserved hepatic conjugation capacity and patent biliary excretion, supporting the interpretation that observed AST elevations represent mild, localized hepatocellular injury without progression to hepatic insufficiency or cholestasis (Giannini et al., 2005). This pattern suggests that the liver retains adequate functional reserve despite biochemical evidence of cellular stress.

4.8 Electrolyte Balance and Acid-Base Homeostasis

The observed alterations in serum electrolytes reduces sodium, increased chloride, and elevated bicarbonate suggesting subtle perturbations in fluid and electrolyte homeostasis and acid-base balance. Sodium, the primary extracellular cation, plays critical roles in maintaining osmotic pressure, nerve impulse transmission, and muscle contraction (Palmer and Clegg, 2017). Hyponatremia can result from dilutional effects, renal sodium wasting, or syndrome of inappropriate antidiuretic hormone secretion (SIADH) (Adrogué and Madias, 2012). The reduction reported by Fisher (1969) in similar contexts suggests that waterborne contaminants might affect renal tubular sodium handling or stimulate antidiuretic hormone release.

Elevated chloride and bicarbonate levels, as documented by Shirley et al. (2003), could indicate compensatory mechanisms to maintain electroneutrality and acid-base balance. The kidneys regulate acid-base homeostasis through bicarbonate reabsorption in proximal tubules and hydrogen ion secretion in distal tubules (Palmer and Clegg, 2017). Increased bicarbonate might reflect metabolic alkalosis or compensatory responses to respiratory acidosis, though blood pH measurements would be necessary to definitively characterize the acid-base status.

Heavy metals can disrupt renal tubular transport mechanisms through inhibition of Na^+-K^+-ATPase, interference with ion channels, and oxidative damage to tubular epithelial cells (Barbier et al., 2005). Cadmium, for instance, accumulates in renal proximal tubules and can cause Fanconi syndrome characterized by generalized proximal tubular dysfunction with wasting of glucose, amino acids, phosphate, and bicarbonate (Järup et al., 1998). However, the relatively mild electrolyte alterations observed in this study, without evidence of severe tubular dysfunction, suggest subclinical effects rather than overt nephrotoxicity.

4.9 Water Hyacinth Biosorption: Efficacy and Limitations

The comparison between untreated and water hyacinth-treated dam water groups reveals important insights into the efficacy and limitations of phytoremediation. The water hyacinth-treated group exhibited several hematological and biochemical parameters similar to those receiving untreated dam water, including leukopenia, elevated neutrophils, and thrombocytosis. This suggests that while water hyacinth demonstrates heavy metal biosorption capacity documented in numerous studies (Priya et al., 2021; Mahamadi, 2022; Rezania et al., 2023), the biosorption process may not completely eliminate toxicological risks.

Several factors could explain the persistent biological effects in water hyacinth-treated water. First, biosorption efficiency varies depending on metal speciation, pH, temperature, contact time, and biosorbent dosage (Mishra and Maiti, 2017; Kumar et al., 2023). The experimental conditions employed (200 g/L biomass, 72-hour contact time, ambient temperature) may not have achieved complete metal removal. Second, water hyacinth biosorption shows differential affinity for various heavy metals; while it efficiently removes certain metals (e.g., Pb, Cd, Cr), others may be less effectively sequestered (Priya et al., 2021). Third, the dam water likely contains complex mixtures of contaminants including organic pollutants, pesticides, and microplastics that are not effectively removed by water hyacinth and may contribute to observed toxicity (Ndimele et al., 2021).

Additionally, the biosorption process might release plant-derived secondary metabolites or alter water chemistry in ways that introduce new concerns. Water hyacinth contains various bioactive compounds including alkaloids, phenolics, and tannins that could leach into treated water and exert biological effects (Shanab et al., 2022). Furthermore, the decomposition of plant material during prolonged contact could release organic matter, alter dissolved oxygen, and modify pH, potentially affecting water quality beyond simple heavy metal removal (Aloo et al., 2023).

4.10 Concentration-Dependent Effects and Dose-Response Relationships

The concentration-dependent nature of observed effects provides valuable insights into exposure-response relationships and potential threshold concentrations. The finding that 20% dam water produced minimal hematological alterations while 40-100% concentrations induced significant leukopenia suggests the existence of concentration thresholds below which adverse effects are negligible or absent. This dose-response relationship supports the concept of hormesis, wherein low-level exposures might stimulate adaptive responses without causing frank toxicity, while higher exposures overwhelm homeostatic mechanisms (Calabrese and Baldwin, 2002).

However, the non-monotonic dose-response observed in several parameters, particularly WBC counts and platelet numbers, deviates from classical toxicological models. The more pronounced leukopenia at intermediate concentrations (60-80%) compared to the highest concentration (100%) exemplifies this complexity. Such non-linear relationships are increasingly recognized in toxicology and can result from receptor-mediated effects, metabolic saturation, competing toxicokinetic processes, or interactions among multiple contaminants (Vandenberg et al., 2012; Zhou et al., 2023). These findings underscore the importance of testing multiple concentration ranges rather than assuming linear dose-response relationships.

4.11 Public Health and Environmental Implications

The findings of this study carry significant implications for water resource management, phytoremediation applications, and public health policy. The observed hematological and biochemical alterations, while generally mild and within acceptable physiological ranges, raise important questions about the long-term safety of utilizing dam water for domestic, agricultural, and aquaculture purposes. Chronic low-level exposures to waterborne contaminants, even at concentrations that do not produce overt toxicity in acute or subacute studies, can accumulate over time and lead to adverse health outcomes including immunosuppression, carcinogenesis, reproductive dysfunction, and developmental abnormalities (Tchounwou et al., 2012).

The leukopenia observed across multiple treatment groups is particularly concerning from an immunological perspective. Individuals with compromised immune function—including children, elderly persons, and immunosuppressed patients might be especially vulnerable to infections and reduced vaccine responses when exposed to immunosuppressive contaminants (Dietert and Piepenbrink, 2006). Moreover, subclinical immune dysfunction could predispose populations to increased disease susceptibility without manifesting obvious symptoms, making detection and intervention challenging.

From a phytoremediation perspective, these results support the potential utility of water hyacinth in heavy metal biosorption while highlighting the necessity for optimization and quality assurance. The fact that water hyacinth-treated water still produced biological effects comparable to untreated water suggests that current biosorption protocols may require refinement. Factors deserving investigation include optimization of biosorbent dosage, contact time, pH adjustment, sequential or continuous treatment systems, and combination approaches integrating phytoremediation with conventional treatment methods (Ali et al., 2023; Pandey et al., 2024).

4.12 Comparative Analysis with Previous Studies

The current findings show both concordance and divergence from previous investigations in this domain. The absence of erythrocyte alterations aligns with studies by Delwatta et al. (2018) establishing reference hematological values for rats and reports by Jahn (1988) on water treatment safety. However, the observed leukopenia contrasts with some studies reporting no immunological effects while agreeing with others documenting immunosuppression following environmental contaminant exposure (Olorunisola et al., 2012; Hemdan et al., 2006).

The hepatic enzyme elevations, particularly AST, corroborate findings by Yang et al. (2014) and Aleman et al. (1998) regarding hepatotoxicity markers, while the preserved bilirubin metabolism differs from studies reporting cholestatic injury patterns. These variations likely reflect differences in contaminant profiles, exposure durations, animal models, and analytical methodologies. The complex interplay of these factors emphasizes the importance of site-specific assessments rather than extrapolating findings across different water sources.

Recent investigations into heavy metal toxicity have employed advanced methodologies including omics approaches (genomics, proteomics, metabolomics) to elucidate molecular mechanisms of toxicity (Adeyemi and Faniyan, 2023; Bhattacharya, 2022). Such approaches could provide deeper insights into the molecular pathways underlying the hematological and biochemical alterations observed in this study. Future research integrating traditional toxicological parameters with molecular biomarkers would enhance mechanistic understanding and risk assessment capabilities.

4.13 Study Limitations and Future Directions

Several limitations should be acknowledged in interpreting these findings. First, the 28-day exposure duration, while appropriate for sub-acute toxicity assessment, does not capture potential chronic effects that might emerge with prolonged exposure. Chronic toxicity studies (90 days or longer) and multigenerational reproductive toxicity studies would provide more comprehensive safety data. Second, the study did not include quantification of specific heavy metal concentrations in dam water before and after water hyacinth treatment, limiting mechanistic interpretations. Future investigations should incorporate comprehensive chemical analysis including speciation studies to correlate biological effects with specific contaminants.

Third, the absence of histopathological examination represents a significant limitation. Microscopic evaluation of liver, kidney, spleen, and bone marrow would provide crucial insights into tissue-level alterations that might not manifest in circulating biomarkers. Histopathology could reveal subtle changes such as hepatocellular vacuolation, tubular degeneration, splenic congestion, or bone marrow hypoplasia that precede functional impairment. Fourth, the study did not assess oxidative stress markers (malondialdehyde, glutathione, superoxide dismutase, catalase) or inflammatory cytokines (TNF-α, IL-1β, IL-6), which could elucidate mechanisms underlying observed toxicity.

Future research should also investigate: (1) bioaccumulation patterns of heavy metals in various tissues; (2) genotoxic effects through comet assay, micronucleus test, and chromosomal aberration analysis; (3) immunological function through lymphocyte proliferation assays, cytokine profiling, and natural killer cell activity; (4) optimization of water hyacinth biosorption parameters including biosorbent pretreatment, pH modification, and sequential treatment systems; (5) economic feasibility and scalability of water hyacinth-based phytoremediation for large-scale water treatment; and (6) environmental fate of harvested water hyacinth biomass containing accumulated heavy metals to prevent secondary pollution.

Additionally, field studies evaluating the health status of human populations and livestock consuming Asa Dam water would provide valuable real-world data complementing laboratory animal studies. Epidemiological investigations could assess associations between water source and disease prevalence, immune function, growth parameters in children, and reproductive outcomes. Such translational research would bridge the gap between experimental toxicology and public health interventions.

4.14 Integration and Synthesis

In synthesis, this investigation demonstrates that Asa Dam water, both untreated and following water hyacinth biosorption treatment, produces concentration-dependent hematological and biochemical alterations in rats. The preservation of erythrocyte parameters and absence of anemia represent encouraging findings, suggesting minimal erythrotoxicity. However, the observed leukopenia, monocytosis, hepatic enzyme elevations, and electrolyte imbalances warrant careful consideration, particularly for vulnerable populations and long-term exposure scenarios. Water hyacinth biosorption, while demonstrating promise as a low-cost, eco-friendly remediation strategy, requires optimization to achieve complete detoxification. These findings contribute to the growing body of evidence supporting phytoremediation while emphasizing the critical importance of comprehensive safety evaluations before widespread implementation in water treatment systems.

V. CONCLUSION

This study comprehensively evaluated the toxicological profile of Asa Dam water and water hyacinth-treated dam water through hematological and biochemical analysis in *Rattus norvegicus*. The key findings demonstrate that while erythrocyte parameters remained within normal physiological ranges across all treatment groups, indicating absence of anemic conditions, significant alterations were observed in immune cell populations and hepatorenal biochemical markers.

The concentration-dependent leukopenia observed in groups receiving 40-100% dam water and water hyacinth-treated water raises important concerns regarding immunological competence and host defense mechanisms. The significant monocytosis in higher concentration groups may represent compensatory responses to environmental stress or subclinical inflammatory processes. Platelet count variations, characterized by thrombocytosis at intermediate concentrations and thrombocytopenia at the highest concentration, suggest complex dose-response relationships requiring further investigation.

Biochemical analysis revealed selective hepatic enzyme elevations and increased serum globulin without overt hepatic insufficiency, as evidenced by preserved albumin synthesis and bilirubin metabolism. Renal function markers showed dissociation between elevated urea and maintained creatinine, suggesting enhanced protein catabolism or metabolic stress rather than glomerular dysfunction. Electrolyte imbalances, including reduced sodium and elevated chloride and bicarbonate, indicate subtle perturbations in renal tubular function and acid-base homeostasis.

Critically, the water hyacinth-treated group exhibited hematological and biochemical profiles comparable to untreated dam water groups, suggesting that biosorption under the experimental conditions employed did not achieve complete detoxification. This finding highlights both the potential and limitations of phytoremediation approaches. While water hyacinth demonstrates established heavy metal biosorption capacity, optimization of treatment parameters including biosorbent dosage, contact time, pH conditions, and sequential treatment systems may be necessary to enhance efficacy and ensure toxicological safety.

From a public health perspective, these findings advocate for cautious interpretation and application. The minimal erythrotoxic effects provide reassurance regarding anemia risk, but the immunological and hepatorenal alterations warrant ongoing monitoring, particularly for vulnerable populations and long-term exposure scenarios. The results support the integration of water hyacinth phytoremediation into comprehensive water treatment strategies while emphasizing the necessity for quality assurance, optimization, and multi-barrier approaches to ensure water safety.

Future research directions should encompass: (1) chronic toxicity studies with extended exposure durations; (2) comprehensive chemical characterization of water before and after treatment;

(3) histopathological examination of target organs; (4) molecular biomarkers of oxidative stress and inflammation; (5) genotoxicity assessment; (6) optimization of biosorption protocols; and (7) field studies in exposed populations. Such investigations will provide the robust evidence base necessary for informed decision-making regarding phytoremediation implementation and water resource management.

In conclusion, water hyacinth-mediated phytoremediation represents a promising, sustainable approach to heavy metal remediation in aquatic systems. However, the biological effects observed in this study underscore the critical importance of comprehensive safety evaluations, process optimization, and integrated treatment approaches. The preservation of erythrocyte integrity alongside immunological and biochemical alterations presents a nuanced toxicological profile requiring careful consideration in translating phytoremediation from research to practical application. These findings contribute valuable insights to the growing knowledge base on phytoremediation safety and inform evidence-based approaches to sustainable water management.

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