# Biochemical and Histopathological Alteration in Cyprinus Carpio Exposed to CuSO4 and Died with Azolla and Duckweed

**التغيرات الكيموحيوية والنسيجية المرضية في أسماك الكارب والمعاملة بكبريتات النحاس والمغذاة على الأزولا وعدس الماء**

By


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# Biochemical and Histopathological Alteration in *Cyprinus Carpio* Exposed to $CuSO_4$ and Died with Azolla and Duckweed

**Abstract :**

Developing suitable materials for fish to prevent heavy metal pollution in water is a delicate topic in fish farming. The effect of adding Azolla and Duckweed to the diet of carp (*Cyprinus carpio*) and its involvement in coping with copper toxicity were studied in this study. In the experiment, carp (*Cyprinus carpio*) divided into 5 groups. The first group left untreated (negative control), second group as the positive control treated with copper sulfate $CuSO_4$ only at a concentration of (0.5 mg/L), the third group was treated with (0.5 mg/L) $CuSO_4$ with Azolla, the fourth group used $CuSO_4$ (0.5 mg/L) in duckweed plant water, while the fifth group used $CuSO_4$ (0.5 mg/L) in Azolla and duckweed mixture. The treatments lasted 56 days. Each group of treated fish had their overall clinical complaints assessed. At the end of the 56-day period, the fish from each group were autopsied. Alanine-aminotransferase (ALT), aspartate-aminotransferase (AST), urea, uric acid and creatinine levels were measured. The result reflected different clinical symptoms in common carp. The blood parameters revealed significant increase in the second group treated with copper sulfate and the significant decrease were in the fifth group. The degree of histopathological alterations and lesions of the liver and kidney differed depending on the Azolla-aquatic duckweed plant mixture treated with $CuSO_4$. According to this study, Azolla and duckweed can be utilized in fish diets to minimize the harmful effects of copper.

**Keywords:** *Cyprinus Carpio*, Azolla, Duckweed, $CuSO_4$

**المستخلص:**

يعد تطوير المواد المناسبة للأسماك لمنع تلوث المياه بالمعادن الثقيلة موضوعًا حساسًا في تربية الأسماك. تمت دراسة إضافة نبات الأزولا Azola وعدس الماء Duckweed إلى عليقة أسماك الكارب (*Cyprinus carpio*) ومدى تأثيرها في التغلب على سمية النحاس. قسمت أسماك الكارب (*Cyprinus carpio*) إلى ٥ مجموعات. تركت المجموعة الأولى دون معالجة (السيطرة السالبة)، وعُوملت المجموعة الثانية بكبريتات النحاس CuSo4 فقط بتركيز (٠.٥ ملغم/لتر)، وعوملت المجموعة الثالثة بكبريتات النحاس (٠.٥ ملغم/لتر) مع الأزولا. عُوملت المجموعة الرابعة بكبريتات النحاس (٠.٥ ملغم/لتر) مع نبات عدس الماء، بينما عُوملت المجموعة الخامسة بكبريتات النحاس (٠.٥ ملغم/لتر) مع خليط الأزولا وعدس الماء. استمرت مدة البحث ٥٦ يومًا. تم ملاحظة العلامات السريرية لكل المجاميع وفي نهاية التجارب تم تشريح الأسماك وجمع العينات النسيجية للكبد والكلية. تم قياس مستويات انزيمات الألانين - ناقل الأمين (ALT) والأسبارتات - ناقل الأمين (AST) واليوريا وحامض البوليك والكرياتينين. اظهرت النتائج زيادة معنوية في هذه المؤشرات في دم اسماك الكارب في المجموعة الثانية المعاملة بكبريتات النحاس والمستوى الأقل معنوية كان في المجموعة الخامسة. اختلفت درجة التغيرات والآفات النسيجية المرضية للكبد والكلية اعتمادًا على خليط نبات الأزولا وطحلب البط المُعالج لكبريتات النحاس والتي اظهرت تحسنا بالتغيرات المرضية النسيجية. وفقا لهذه الدراسة يمكن استخدام الأزولا وعدس الماء في النظام الغذائي للأسماك لتقليل الآثار الضارة للنحاس.

**الكلمات المفتاحية:** اسماك الكارب الشائع، أزولا، عدس الماء، كبريتات النحاس

## Introduction

When feeding costs more than half the cost of tasks, protein is the most expensive element in specially designed diets in intensive aquaculture. Fish meal (FM) is the costliest form of amino in both aquaculture meals and a variety of animal diets due to its well-known nutritional profile and high palatability. (1). Fish is one of the primary protein sources in aquaculture zones, and it contributes significantly to both food safety and economic development. According to experts, aquaculture has the best possibility of meeting the growing demand for aquatic food. It is the fastest-growing segment of the food industry (2).



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Poor management, toxicity, and elevated heavy metal levels, on the other hand, damage fish immune systems and harm the aquatic ecology, promoting bioaccumulation in fish tissue, and exposing fish to a variety of disease outbreaks (3).

Cottonseed meal, meal from soybeans, oil from ground nuts, and mustard oil cake are all examples of oil that is edible. are common ingredients in formulated feeds, are in low supply, aquafeed makers and fish farmers do not have easy access to them. It is apparent that underdeveloped countries cannot rely on FM as the The primary sources of protein in aquafeeds. This has hampered the development and cost of aquaculture in the majority of underdeveloped countries (4). Growing industrial, agricultural, and commercial activity produces major environmental problems and has an influence on the species that dwell in contaminated environments (5).

Animals in terrestrial and aquatic settings are frequently exposed to heavy metal pollution, which can occasionally cause serious health problems such as cancer. Heavy metals accumulate in the tissues of living creatures because they are not physiologically degradable (6). Copper constitutes one of the most significant heavy metal contaminants affecting aquatic life (7). Several studies have found that copper exposure reduces fish body weight and inhibits lipase and digestive enzymes (8). Furthermore, human copper toxicity has been linked to hyperactivity, malignancy, anemia, brain and liver damage, and renal failure (9).

Azolla is a freshwater floating plant that generates a lot of biomass and protein. It can be used directly in fish feed or as a supplement to a different protein source in the diet. Azolla's value in aquaculture has expanded due to its higher crude protein content (13% to 30%) and key amino acid composition (EAAs),


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which is high in lysine when compared to other green feed crops and aquatic macrophytes. (10). Duckweed belongs to a small family of floating aquatic plants. With 40 species identified to date, there are four genera: Lemna, Spirodela, Wolfilla, and Wolffiella. Lemna minor is the most important Lemna species. It is significantly less expensive than other plant protein sources and may be manufactured in huge quantities at a reasonable cost. Duckweed is gaining popularity as a high-protein (40-45% dry weight) fish food. Duckweed protein is more similar to animal protein in that way, with higher amounts of the essential amino acids lysine and methionine than other plant proteins (11). In the current study, blood biochemical parameters of common carp exposed to copper sulfate were evaluated to determine the effects of azolla.

**Materials and Method**

**Design of Study**

Mosul University's Fisheries Faculty of Agriculture conducted the study. For 56 days, the studies were conducted in a circular plastic tank at an average temperature of 22.8, and a pH of 8.1$\pm$ 0.3. Five experimental groups of 125 common carps were formed, with a mean weight of 70.2 +/- 0.4 g:

- **Group 1:** untreated (negative control).
- **Group 2:** positive control (treatment with copper sulfate $CuSO_4$ alone) for 56 days at a concentration of (0.5 mg/L).
- **Group 3:** $CuSO_4$ treatment with Azolla (0.5 mg/liter) for 56 days.
- **Group 4:** $CuSO_4$ treatment (0.5 mg/liter) in Duckweed plant water for 56 days.
- **Group 5:** $CuSO_4$ treatment (0.5 mg/liter) with an Azolla and Duckweed for 56 days. Gross clinical signs were investigated in the entire fish population of each treatment.



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**Sampling**

After centrifuging blood after spinning the samples at 3000 rotations per minute for a period of ten minutes, the serum was extracted. using the Vitros System Chemistry 350. The serum was then refrigerated at -20 °C until the assays were performed.

**Fertilizer proliferation**

The Azolla species are replicated (dechlorinated) in a pond with some mild shade and tap water. 70 g of sheep excrement were dissolved in 5 days' worth of tap water to generate fertilizer. The greenery was then moved to the pond. The forming material went away after seven days.. Allowing it to swing and dry in the air for at least two days before storing it in a plastic bag (12).

**Duckweed cultivation**

200 square meters (20 m 10 m) concrete ponds were ready for duckweed cultivation. The pond's bottom was meticulously cleaned before adding ground water (50 cm). Cattle dung, bird droppingsAt a rate of 1.052 Kg/m3,, and mustard oil on a cake (1:1:1) received treatment. Based on the results of outside concrete tanks, organic manures were chosen to duckweed cultivation. The pond was used to simulate the L. minor culture conditions established in the tank trials. Later, fresh duckweed was grown in a cement tank at Mosul University's agriculture campus at a rate of 1 kilogram per pond. It just covered a small area of the pond. Harvesting began once the plant had entirely engulfed the pond's surface (13).

**Biochemical parameter analysis**

Using a veterinarian biochemical analyzer (VS2 Abaxis, USA), blood enzymes such as aminotransferase aminotransaminase (ALT), aspartate aminotransaminase (AST),


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as well as others had been identified in serum urea, uric acid, and creatinine.

**Histopathological examination**

At random, five groups of 125 carp fish were produced. After a 56-day experiment, the fish were slaughtered so that samples of their liver and kidney could be collected for rotational histological analysis(١٤)

**Statistical investigation**

To generate cross-tabs and obtain the relevant findings, data were collected, processed, and statistically analyzed using the SPSS statistical program version 26. The observed data were tabulated, and the variable groups were assessed with a one-way ANOVA and an Duncan test. The *P*-value result was judged significant when it was less than 0.05; when it was larger than 0.05, it was considered non-significant. The mean and standard error were used to express the effects of continuous variables

**Results**

The levels for ALT, AST, urea, uric acid, urea, and creatinine in the study groups are shown in Table 1. The results showed that Group 2 (copper sulfate) had a substantially higher ALT of 29.646 2.335, followed by Group 5 (copper sulfate with a mixture of azolla and Duckweed) and Group 3 (copper sulfate with Azolla) of 15.574 1.029 and 15.524 0.491, respectively. Group 4 (copper sulfate with water Duckweed plant) had the lowest levels of ALT at 14.88 0.968. Refer to Table 1. The AST level was greater in Group 2 (copper sulfate) 177.94 8.398 compared to Group 1 (controls) 90.148 4.834. Group 3 (copper sulfate with Azolla), Group 4 (copper sulfate with water Duckweed plant), and Group 5 (copper sulfate with azolla and Duckweed mixture) 86.82 4.473, 81.976 3.080, and 75.956 4.230, respectively. Furthermore, the results suggest that urea levels are much higher in Group 2 (copper sulfate) 8.596 0.562, followed by Group 5 (copper sulfate with azolla and Duckweed mixture) and Group 4 (copper sulfate with water Duckweed plant) 6.112 0.452,



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5.458 0.282, respectively. Group 3 (copper sulfate with Azolla) had the lowest urea level of 2.384 0.181 (Table 1). Uric acid levels were higher in Group 2 (copper sulfate), followed by Group 3 (copper sulfate with Azolla) 26.82 1.527, Group 5 (copper sulfate with a mixture of azolla and Duckweed) 22.204 2.030, and Group 4 (copper sulfate with water Duckweed plant) 20.724 2.243 compared to Group 1 (controls) 17.532 0.926. Furthermore, the results revealed that Creatinine was considerably higher in Group 2 (copper sulfate) 288.54 3.964, followed by Group 3 (copper sulfate with Azolla) 34.38 4.135. Creatinine levels were lower in Group 4 (copper sulfate with water Duckweed plant) and Group 5 (copper sulfate with a mixture of azolla and Duckweed) than in Group 1 (controls) at 27.142 1.090 and 18.142 0.503, respectively. Referring to Table 1.

**Table 1: ALT, AST, Urea, Uric acid, and Creatinine levels varied between research groups.**

| Parameters | ALT | AST | Urea | Uric acid | Creatinine |
|---|---|---|---|---|---|
| Study groups | Mean ±St. error | | | | |
| Group 1<br>Controls | 14.102 ±1.101<br>B | 90.148<br>±4.834<br>B | 2.268 ±0.190<br>C | 17.532<br>±0.926<br>B | 31.04 ±1.865<br>B |
| Group 2<br>copper sulfate<br>CuSo4 | 29.646 ±2.335<br>A | 177.94<br>±8.398<br>A | 8.596 ±0.562<br>A | 60.742<br>±3.208<br>A | 88.54 ±3.964<br>A |
| Group -3-<br>copper sulfate with Azolla | 15.524 ±0.491<br>B | 86.82 ±4.473<br>B | 2.384 ±0.181<br>B | 26.82 ±1.527<br>B | 34.38 ±4.135<br>B |
| Group- 4-<br>copper sulfate with water Duckweed plant | 14.88 ±0.968<br>B | 81.976<br>±3.080<br>B | 5.458 ±0.282<br>B | 20.724<br>±2.243<br>B | 27.142<br>±1.090<br>B |
| Group 5<br>copper sulfate with a mixture of azolla and Duckweed | 15.574 ±1.029<br>B | 75.956<br>±4.230<br>B | 2.112 ±0.452<br>C | 22.204<br>±2.030<br>B | 18.142<br>±0.503<br>C |
| *P*-value | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |

Data expressed as Mean ± stander error (N= ٤ animals)


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Different letters among groups in rows mean there is significant difference at $p \leq 0.05$

The following observations were made using the histological damage score and histological section analysis. Histopathological tests indicated severe structural abnormalities in the livers of fish that had received repeated treatments with different extracts. The liver of a fish from the CuSO4 treatment group was histologically examined in this study. This indicated bile duct epithelial cells, considerable congestion, and edema in the portal region (figure 1). Figures 2 and 4 show sinusoid dilatation, edema in pancreatic tissue, congestion with vacuolar degeneration, fibrosis, and fat deposition (figure 4).

Histological examination of C.carpio fish liver treated with CuSO4 and Azolla indicated congestion and vacuolar degeneration (figure 5). Furthermore, the studies demonstrated that CuSO4-treated fish liverOn the other hand, histological investigation of C.carpio fish liver treated to CuSO4 with Azolla revealed congestion and vacuolar degeneration (figure 5). Furthermore, fish liver treated with CuSO4 and a mixture of azolla and Duckweed water plant dilatation of sinusoid and vacuolar degeneration (figure 6).



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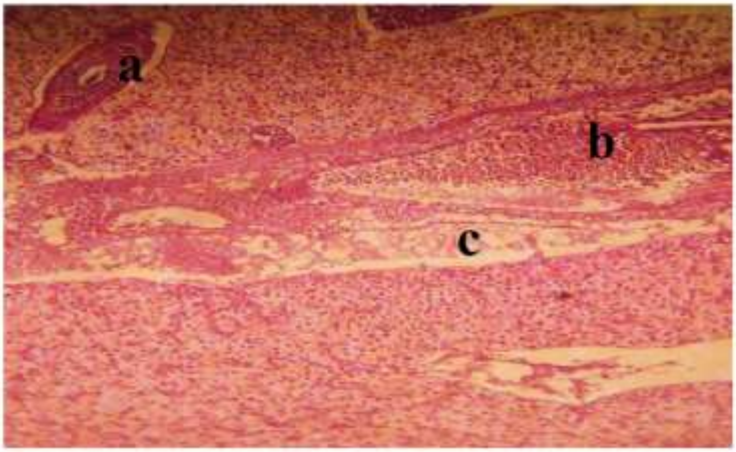
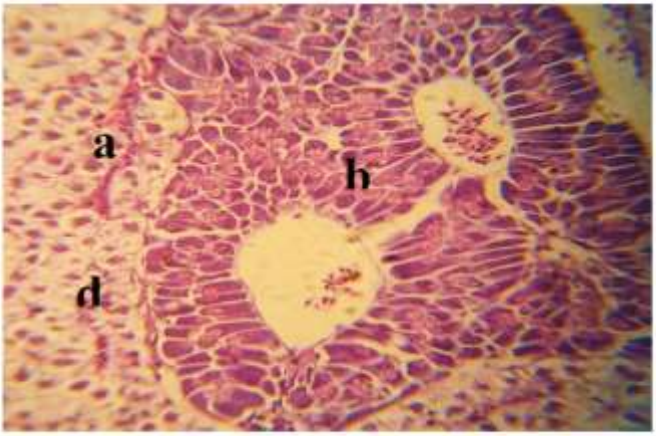



**Figure(1): Microscopic examination of liver in *C.carpio* exposed to $CuSO_4$ for 56 day show hyperplasia of epithelial cells lining bile duct (a), severe congestion (b) edema in the portal area (c), H&E, 10 * 3X**




**Figure(2): Microscopic examination of liver in *C.carpio* exposed to CuSO4 for 56 day show dilatation of sinusoid (a), edema in pancreatic tissue(b) congestion (c) with vacuolar degeneration(d), H&E, 40 * 1.8X.**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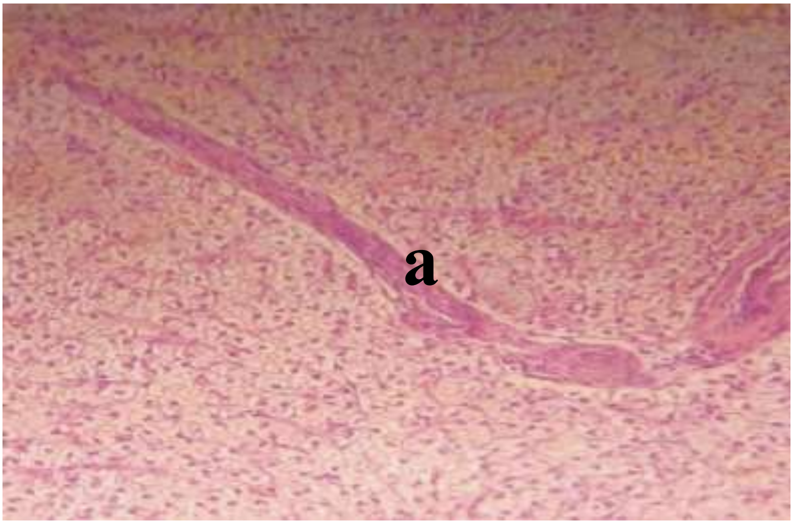
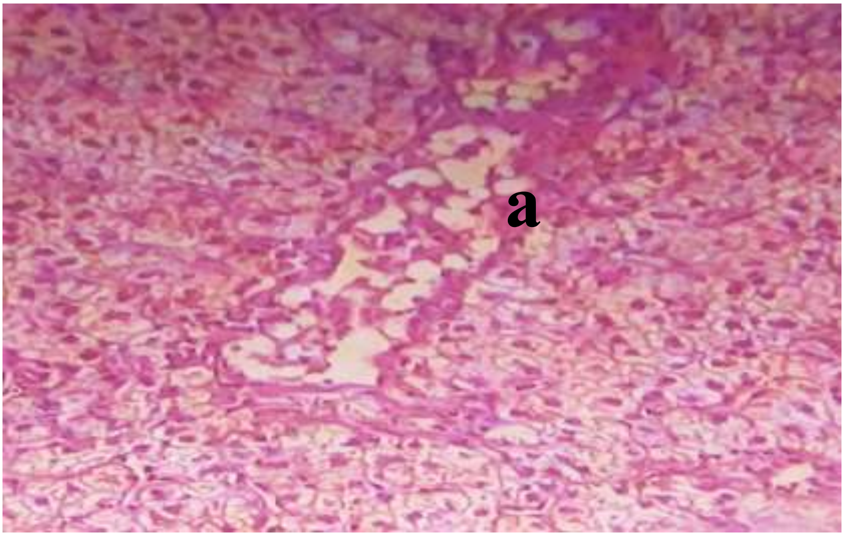



**Figure(3) :Microscopic examination of liver in *C.carpio* exposed to $CuSO_4$ show fibrosis (a),H&E, 10 *6.3 X.**




**Figure(4) Microscopic examination of liver in *C.carpio* exposed to $CuSO_4$ for 56 day show fat deposition (a),H&E, 10 *8.8X.**



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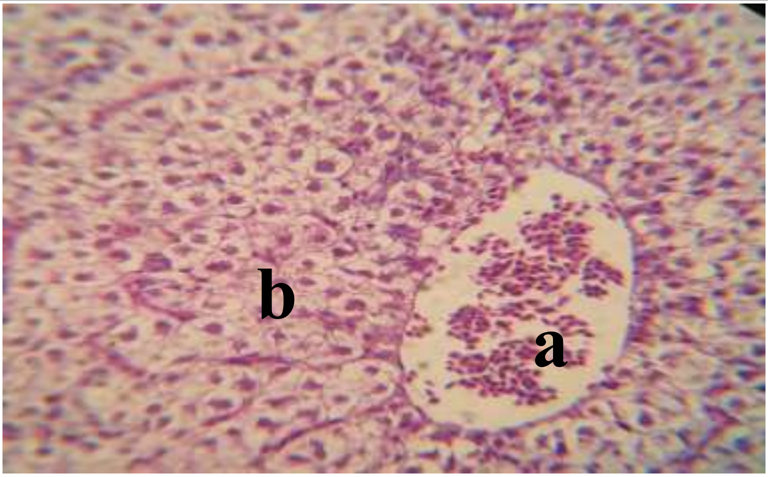
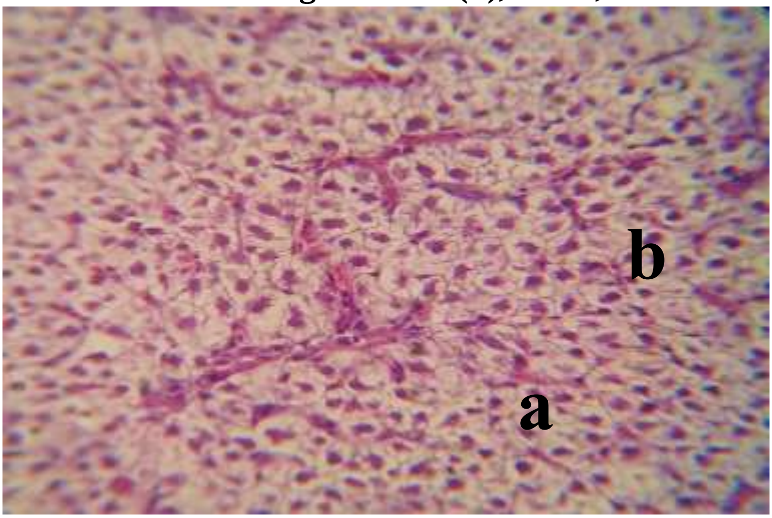



**Figure(5): Microscopic examination of liver in *C.carpio* exposed to $CuSO_4$ with Azolla for 56 day show congestion (a) and vacuolar degeneration(b), H&E,40*1.8X.**




**Figure(6) Microscopic examination of liver in *C.carpio* exposed to $CuSO_4$ with a mixture of Azolla and Duckweed water plant for 56 day show dilatation of sinusoid (a) and and vacuolar degeneration(b) ), H&E,40*1.8X.**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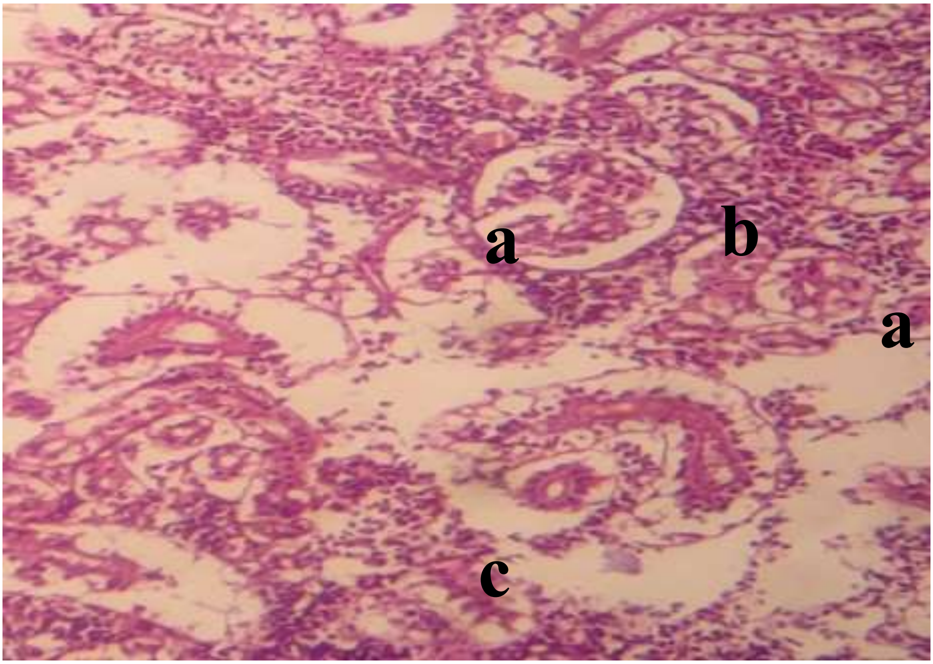
Histological examination of a CuSO4-treated fish's kidney indicated the existence of interstitial nephritis, tubular necrosis, and renal sac occurrence (figure 7). Microscopic analysis of the kidney in C.carpio subjected to CuSO4 and Duckweed water plant reveals interstitial nephritis, hydropic degeneration of the epithelial cell lining the renal tubule, and the presence of renal sacs (figure 8). Renal hyaline cast, hydropic degeneration of epithelial cell lining renal tubule atrophy of glomerular, and heamorraghe were observed in the group exposed to CuSO4 and a mixture of Azolla and Duckweed water plant (figure 9).




**Figure(7) Microscopic examination of kidney in *C.carpio* exposed to $CuSO_4$ for 56 day show interstitial nephritis (a), tubular necrosis(b) and renal sac occurrence (c),H&E, 10*6X.**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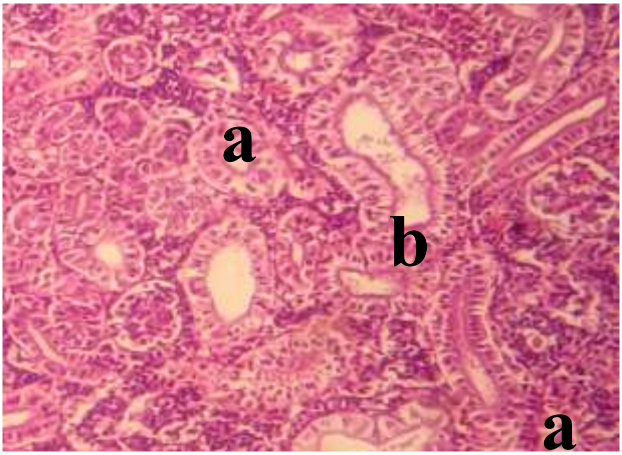
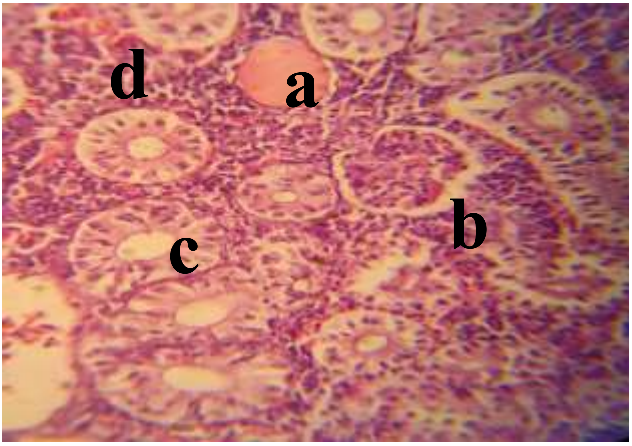



**Figure(8): Microscopic examination of kidney in *C.carpio* exposed to $CuSO_4$ and Duckweed water plant for 56 day show interstitial nephritis (a), hydropic degeneration of epithelial cell lining renal tubule (b) and renal sac occurrence (c),H&E, 10*6X.**




**Figure(9) Microscopic examination of kidney in *C.carpio* exposed to $CuSO_4$ and mixture of Azolla and Duckweed water plant for 56 day show renal hyaline cast (a), hydropic degeneration of epithelial cell lining renal tubule (b) atrophy of glomerular (c), and heamorraghe H&E, 40*2.3X.**



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**Discussions**

As a result, it was determined that copper sulphate had the largest influence on fish. Previous research has shown that Azolla can protect carp fish from the detrimental effects of heavy metal pollution (14). This protective function is assumed to be due to Azolla's high cation capacity. It is well understood that one of the detrimental effects of heavy metal pollution is the suppression of enzyme metabolism. Furthermore, it is well recognized that lipid metabolism, liver and kidney functions, and levels of cholesterol, lipoproteins, and triglycerides are all linked (15). According to reports, adding 2-5% Azolla to cattle feeds enhanced body weight and decreased diarrhea incidence (16). After considering previous findings and current data, it is possible to conclude that using Azolla to combat copper toxicity may protect fish against a variety of detrimental consequences of the metal. As a result, more research into different dosages and other fish species is required. In light of these factors, the outcomes of this study indicate that the Azolla treatment used in this study was effective in protecting fish from a variety of harmful repercussions. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels are considered.

Other studies on fish and fowl fed diets with varying Azolla levels came to similar conclusions (17). Furthermore, it mitigates the effects of copper exposure by eliminating the copper load from the water (18). When dietary Azolla is ingested more regularly, cholesterol begins to grow in the other direction. The liver, kidneys, heart, skeletal muscle, pancreas, spleen, erythrocyte, brain, and gills can all contain ALT and AST (19).


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These blood plasma enzymes are being studied clinically as indications of liver health. Because these enzymes are released into the bloodstream when there is an injury, they may indicate liver dysfunction. In this study, serum ALT and AST levels increased significantly after FM was replaced with Azolla meal. It demonstrates that the liver suffered as a result of the FM substitution with Azolla. Xu et al. (2018) (20) reported that blood ALT and cholesterol levels in C. carpio fed FM replacement meals were significantly lower than those in the control group (21). Furthermore, in this study, the nutritional needs of C. carpio fingerlings were addressed by adding fish meal to Azolla meal. However, the presence of anti-nutritional factors (ANFs) and a high dietary fiber content may be the cause of decreased feed efficiency at higher Azolla meal inclusions, which in turn decreased growth performance (22). Because Azolla has a comparable digestive enzyme profile and a diet rich in -6 fatty acids, it has the potential to replace expensive FM in herbivorous animals (23).

Fish can be exposed to heavy metals in two ways, according to Yousif et al. (2021) (24), either indirectly through their skin and gills or directly through consuming contaminated water or food. It can cause tissue damage even in small amounts, as demonstrated in the colon and liver. Thus, histological abnormalities in the liver and intestines of ordinary people were discovered in our investigation. Toxins such as heavy metals are detoxified by endogenous waste products and externally generated liver as a result of their buildup in fish organs;

consequently, The cumulative influence of metal content in the liver may cause pathological changes in the liver .

The cellular degradation of the liver may be induced by a lack of oxygen, heavy metal effects on the circulatory system, changes in blood vessel permeability, and other factors (25). Duckweed systems have been used to remove contaminants from several types of wastewater, including domestic wastewater, septage, and agricultural wastewater, such as swine water. According to research, these plants improve the overall quality of eutrophic water bodies and aid in heavy metal absorption from contaminated waters. Duckweeds have more potential as a therapy system than most other aquatic plants due to their specific morphological and physiological properties, high nutritional content, and range of reuse prospects (26, 27).

At this time, comprehensive and thorough discussion and comparisons cannot be done due to a lack of study addressing the particular features and implications of Azolla minerals. As a result, more research is needed to determine the biological and metabolic features, as well as the consequences of Azollas in different species.

**Conclusion**

The nutritional value of Azolla meal with duckweed as a component of C. carpio communis fingerling diets was determined in this study. It appears to be a good substitute for fishmeal in real-world diets; with no deleterious effects on C. carpio communis histopathology or serum biochemical indicators. With its expansion, azolla and duckweed meal could be efficiently included to provide cost-effective and


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environmentally friendly feeds for the bulk production of fingerling C. carpio communis.

**Conflicted interested**: None

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