



Qualitative analysis, antioxidant properties of azolla and duckweed against chemo-stress in carp fish exposed to CuSO₄

F.S. Salh¹  and R.T. Altaee² 

¹Department of Basic Sciences, College of Agriculture and Forestry, ²Department of Chemistry, College of Education for Pure Sciences, University of Mosul, Mosul, Iraq

Article information

Article history:

Received 27 February 2025

Accepted 13 August 2025

Published 26 September 2025

Keywords:

Azolla

Duckweed

Cyprinus carpio

CuSO₄

Correspondence:

F.S. Salh

farhsameer@uomosul.edu.iq

Abstract

The study aimed to determine the antioxidant and protective and potential effect of Azolla (*A. filiculoides* Lam.), Duckweed (*Lemna spp.*) and their mixture against the effect of CuSO₄ 0.5 mg/L poisoning on carp fingerlings (*Cyprinus carpio* L.). The study divided in two parts, the first included extraction and identification of the active compounds from Azolla and Duckweed by using HPLC. The second includes the investigation antioxidant potential of Azolla, Duckweed and their mixture against CuSO₄ on carp fingerlings, the experiment consisted of five groups, each group consisting of three replicates over a period of 56 days. The control group: was left (untreated), and the positive control group: treated with CuSO₄ alone 0.5 mg/L. The third group treated with Azolla with CuSO₄, and the fourth group: treated with Duckweed with CuSO₄. Finally, the fifth group: was treated with a mixture of Azolla, Duckweed with CuSO₄. After the end of experimental time 56 days, fish samples were collected, dissected, and organs removed. Significant differences were recorded in the levels of albumin, total protein, triglycerides, cholesterol, and globulin. Pathological changes were also recorded in the tissues of the gills and intestines of fish, which showed hemorrhage with hydropic degeneration of epithelial cells exposed to CuSO₄. The intestine also shows severe hydrophilic degeneration of the epithelial cells covering the villi, with destruction and peeling of the apex of the cells.

DOI: [10.33899/ijvs.2025.157797.4155](https://doi.org/10.33899/ijvs.2025.157797.4155), ©Authors, 2025, College of Veterinary Medicine, University of Mosul.

This is an open access article under the CC BY 4.0 license (<http://creativecommons.org/licenses/by/4.0/>).

Introduction

Rapid industrialization and urbanization have led to heavy metal pollution in freshwater ecosystems, contaminating aquatic food supplies and increasing metal flux from terrestrial sources (1). The presence of heavy metal residue in vegetables grown in polluted areas, as well as fish and shellfish taken from polluted natural sources, raises concerns about health of human (2,3). Many heavy metals (Cu, Zn, Se, Co, Cr and Mn, are required for basic cellular functions), found in freshwater are undesirable and unimportant to aquatic ecosystems. Heavy metals, on the contrary hand, that are generally essential for fish and other aquatic species to grow normally are frequently in excess of

what these aquatic organisms require and end up being harmful to them (4,5). As agricultural output has increased, so has the number of lakes and rivers exposed to potentially hazardous toxins such as copper-containing herbicides. Small wastewater flows can easily convey toxic pollutants into rivers and lakes. Copper sulphate (CuSO₄) is employed commonly as a pesticide in agriculture to combat protozoal and fungal infestation, Copper ions were found at quantities within the permissible limit in a number of freshwater aquatic ecosystems including ground waters (6,7). Copper sulphate is frequently utilized in aquaculture as a potent medication for preventing and treating various ectoparasitic and bacterial diseases (8). Fungi, protozoa, and trematodes are exposure alone. When exposed to copper ions,

Salmonella spp., *Pasteurella spp.*, *Vibrio spp.*, and other bacteria all grown adversely (9). Copper treatment and repeated exposure can significantly increase the amount of this hazardous element in aquaculture ponds (10), lasting harmful for at least 50 hours after one treatment and potentially impairing fish development (11,12). Furthermore, in a catfish farm in Vietnam, the fish have been proven to collect copper (13,14). Azolla is a watery floating plant that produces a significant amount of protein and biomass. It can be used directly in fish feed or as a dietary component for another protein source (15). Azolla has a greater crude protein level 13 to 30% and an essential amino acid (EAA) makeup that is high in lysine when compared with other green crop varieties and aquatic macrophytes (16), beside phyto-constituents such as flavonoids, phenols, alkaloids and saponins (17). Duckweed is a member of the floater family. There are now 40 species classified as belonging to the four genera *Lemna*, *Spirodela*, *Wolffia*, and *Wolffiella*, the most well-known species of *Lemna* is *Lemna minor*. It can be generated in large quantities at a low cost and is far more cost effective than other plant protein alternatives. Duckweed has recently gained popularity as a high-protein (40-45% dry weight) fish food. Duckweed protein is more akin to animal protein in this regard, as it includes more of the necessary amino acids lysine and methionine than other plant proteins, also Duckweed has high levels of flavonoids, minerals, vitamins and fatty acids (PUFAs) (18,19). Blood tests were administered to fish exposed to various water pollutants and toxicants, such as metals, chemicals, pesticides, and industrial effluents, as a sensitive indicator for stress (20). Knowing normal and pathological processes, as well as toxic effects, in fish is now heavily reliant on blood parameter monitoring. Fish blood is utilized in toxicology research and environmental monitoring as a valuable indicator of physiological and pathological changes, aiding scientists in understanding the harm caused by contaminants. Biochemical indicators such as glucose and lipid profile are widely used to assess the toxicity of environmental pollutants (21). Although there have been some histological investigations on the effects of various pollutants on fish kidneys, little is known regarding the influence of copper on the histopathology of exposed fish (22,23).

To identify the effects of Azolla and Duckweed as anti-oxidant aquatic plant, blood biochemical parameters of common carp exposed to copper sulfate were investigated in the current study.

Materials and methods

Ethical approve

The ethical approve was licensed by Ethical Committee in College of Agriculture and Forestry, University of Mosul, dated 01/06/2022 issued CF/UOM/2022/125.

Fertilizer propagation

Azolla species are cultivated in a mildly shaded pond using dechlorinated tap water. Sheep excrement is used to make fertilizer, and the biomass is extracted, dried, then kept in the plastic bag (24).

Cultivation of Duckweed

The concrete ponds of 200 m²/ (20 m×10 m) ready for Duckweed cultivation. Following a thorough cleaning of the pond's bottom, ground water (50 cm) was added. At a rate of 1.052 kg per square meter, cattle dung, bird feces, mustard oil cake (1:1:1) were employed. Duckweed was created using organic manures, chosen based on the results of exterior concrete tanks. The cultivation conditions for (*Lemna spp.*) that were created during the tank trials were adopted in the pond. Eventually, Mosul University's agriculture institution produced new Duckweed in cement tanks at a rate of 1 kilogram per pond. It just partially covered the pond. Harvesting began once the plant had completely covered the pond's surface (25).

Preparation of plants extracts

The Fatty acids were isolated from Azolla and Duckweed using (100gm) of the ground sample placed in a thimble soaked in a Soxhlet apparatus with 500 ml petroleum ether 60-80°C for 48 hours until the color of solvent became clear, using a rotary evaporator, the fatty extract was then packed into sterile bottle and placed in a 2-4°C refrigerator. The oil isolated from Azolla and Duckweed was diagnosed by HPLC technique. Phenolic compounds were obtained from the remaining tissues, were taken and dried to get rid of petroleum ether and the ground sample of the plants (each on at time) placed in a thimble, soaked in a Soxhlet apparatus with 500 ml ethanol (90%) for 48 hours until it became clear. The alcoholic crude extract was obtained after drying through rotary evaporator, the crud extract was kept in an amber vial and placed in the refrigerator, phenolic compounds were identified using HPLC technique (26).

Experimental design

In Mosul University's Fisheries Faculty's College of Agriculture conducted the study. The 56-day test was carried out in a round plastic tank with a pH of 8.1±0.3 and a temperature of 22°C. A total of 125 common carps weighing 71.3±0.5 gm was assigned by random to one of five experiment groups: Group the negative control (untreated) first. Positive control in the second group 0.5 mg/L copper sulfate, CuSO₄. Azolla treatment in Group 3 with a CuSO₄ dose of 0.5 mg/liter. Fourth Group CuSO₄ 0.5 mg/L treatment with Duckweed plant water. CuSO₄ from the Fifth Group and a combination of Azolla and Duckweed. The entire therapy period is 56 days. The entire fish population in each treatment was evaluated for gross clinical symptoms.

Sampling

Blood samples were centrifuged at 3000 rpm for 10 minutes on a Vitros- System –Chemistry- 350 after serum extraction and stored at -20°C for assay preparation.

Analysis of Biochemical parameters

A veterinary biochemistry analyzer was used to test blood enzymes such as total cholesterol (TC), triglyceride (TG), globulin, albumin, and total protein (VS2 Abaxis, USA).

Histopathological analysis

Five groups of 125 carp fish were created at random. After the 56-day treatment, the fish were slain so that samples of their intestines and gills could be obtained for rotational histopathological investigation (27).

Statistical investigation

SPSS version was used to examine the data, using one-way ANOVA and independent t-test to generate cross –tabs and conclusions. The impacts of continuous variables were depicted using mean and standard error.

Results

Qualitative analysis

In the current study fatty acids were diagnosed using HPLC technique, depending on the time of their purity in the separation rod, which in turn depends on the length of the hydrocarbon chain of fatty acid and degree of saturation of fatty acid (Table 1 and Figure 1). The oil extract of both Azolla and Duckweed plants had lowest contents of saturated fatty acids, and the highest of poly unsaturated fatty acids, and monounsaturated fatty acid. The phenolic and flavonoids were also identified by HPLC technique by comparing the retention time of standard compounds (Table 2 and Figure 2).

Table 1: Fatty acids and their percentage in Azolla and Duckweed oil extract

Fatty Acids	Azolla (%)	Duckweed (%)
Palmitic acid	5.25	4.99
Stearic acid	2.69	2.41
Oleic acid	14.58	12.44
Linoleic acid	18.90	16.80
Linolenic acid	1.25	1.05
Arachidonic acid	2.02	1.77

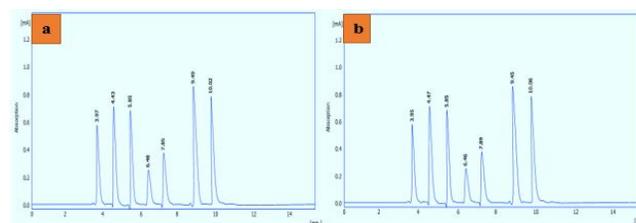


Figure 1: HPLC chromatogram of fatty acids extraction in a) Azolla oil, b) Duckweed oil.

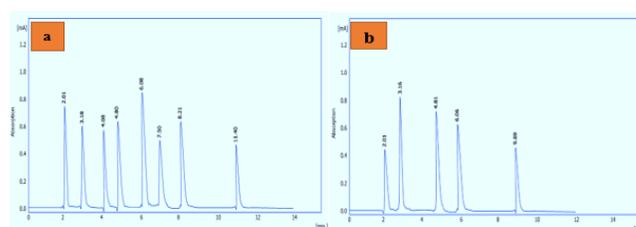


Figure 2: Overlaid HPLC phenolic profile of (a): Azolla (diagnostic compounds: Gallic acid 2.01, Chlorogenic acid 3.16, Caffeic acid 4.81, Ferulic acid 6.08, Ellagic acid 7.50, P-coumaric acids 8.21, Quercetin, 11.40) and (b): Duckweed (Gallic acid 2.01, Chlorogenic acid 3.18, Caffeic acid 4.81, Ferulic acid 6.06, Rutin, 9.89).

Table 2: Compound found in Azolla and Duckweed (alcoholic crude extract)

Peak	Diagnosis of peak	Azolla (µg/gm)	Duckweed (µg/gm)	RT of Standard (min)
1	Gallic acid	22.45	15.81	2.08
2	Chlorogenic acid	18.65	14.77	3.15
3	Caffeic acid	12.66	13.68	4.80
4	Ferulic acid	15.66	10.23	6.00
5	Ellagic acid	19.98	----	7.45
6	P-coumaric acids	13.61	----	8.28
7	Rutin	----	8.98	9.88
8	Quercetin	12.66	----	11.48

Biochemical test

Table 3 shows the concentrations of albumin, total protein, triglycerides, total cholesterol, and globulin in the study groups. The results showed that group 2 (Copper sulfate) had a considerably higher total cholesterol of

6.174±0.304, followed by group 5 (copper sulfate with a mixture of Azolla and Duckweed) and group 4 (copper sulfate with water Duckweed plant) of 3.644±1.618 and 2.936±0.0882, respectively, while group 3 (copper sulfate with Azolla) had reduced total cholesterol levels of

2.726±0.223 compared to controls at 2.884±0.239. Triglyceride levels were greater in group 2 (copper sulfate) 2.772±0.345, followed by group 3 (copper sulfate with Azolla) and group 4 (copper sulfate with water Duckweed plant) (1.098±0.0816, 1.020±0.164), respectively. while group 5 (copper sulfate with Azolla and Duckweed combo) 0.922±0.166 compared to group 1 (controls) 0.944±0.101. In addition, total protein levels were significantly higher in group 2 (copper sulfate) 33.4±1.023, followed by group 4 (copper sulfate with water Duckweed plant), group 5 (copper sulfate with a mixture of Azolla and Duckweed), and group 3 (copper sulfate with Azolla) 29.004±3.235, 28.86 0.697, and 24.598±1.027, respectively, compared to controls. The

levels of albumin were found to be greater in group 2 (copper sulfate). As well as lower in the fifth group (copper sulfate with a combination of Azolla and Duckweed), with 10.564±0.548, 10.134±0.664, and 9.95±0.169, accordingly, compared to group 1 (controls) at 11.334±0.677. Furthermore, the results revealed significantly higher of albumin, the results revealed that higher level of albumin in group 2 (copper sulfate) 21.296±0.846, followed by group 4 (copper sulfate with water Duckweed plant), group 5(copper sulfate with a mixture of Azolla and Duckweed) and group 3(copper with Azolla) 18.902±3.083, 18.29±1.090, 14.464±0.78 respectively compared to group 1 (controls) 11.334±0.928 (Table 3).

Table 3: Triglyceride, Total protein, Albumin, Total cholesterol, and Globulin levels among study groups

Groups	Total Cholesterol	Triglyceride	Total Protein	Albumin	Globulin
Group 1	2.884±0.239 A	0.944±0.101 A	22.52±0.719 A	11.186±0.677A	11.334±0.928 A
Group 2	6.174±0.304 B	2.772±0.345 B	33.4±1.023 B	12.104±0.515 A	21.296±0.846 B
Group 3	2.726±0.223 A	1.098±0.0816 A	24.598±1.027 A	10.134±0.664 A	14.464±0.781 A
Group 4	2.936±0.088 A	1.020±0.164 A	29.004±3.235 C	9.95±0.169 A	14.464±0.781 A
Group 5	3.644±1.618 A	0.922±0.166 A	28.86±0.697 C	10.564±0.548 A	18.29±1.090 A
Total	3.6728	1.3512	27.6764	10.7876	16.8572
P value	0.001	0.001	0.001	0.001	0.001

Total Cholesterol (mmol/L), Triglyceride (mmol/L). Total protein (gm/L). Albumin (gm/L). Globulin (gm/L). *Data as Mean ± slandered errors. P<0.05.

Histopathological result

The following conclusions were drawn from the examination of histological sections and the histological damage score. Histopathological examinations revealed that fish that had been repeatedly exposed to various extracts had severe structural abnormalities in their gills and intestine. The gills of a fish from the CuSO₄ treatment group in this study were investigated histologically. This demonstrated that *C. carpio* gill subjected to CuSO₄ for 56 days exhibits hyperplasia of mucus cells with edema and sloughing of epithelial cells (Figure 3). A microscopic study of the gills of *C. carpio* subjected to CuSO₄ reveals hemorrhage as well as hydropic degeneration of epithelial cells (Figure 4). A microscopic analysis of the gill in *C. carpio* subjected to CuSO₄ and Duckweed reveals normal interlamellar space (Figure 5). On the other hand, histological examination of *C. carpio* kidney subjected to CuSO₄, Azolla, and Duckweed revealed loss of straight secondary gill filaments as well as hydropic degeneration of epithelial cells (Figure 6).

A histological examination of the gut of a CuSO₄ treated fish reveals severe hydropic deterioration of epithelial cells lining the villi, as well as damage and sloughing of the villi apex (Figure 7). Furthermore, a microscopic examination of the gut of *C. carpio* subjected to CuSO₄ reveals infiltration of inflammatory cells with adhesion between villi, as well as extensive hydropic deterioration of epithelial cells (Figure 8). Furthermore, the intestine of *C. carpio* subjected to CuSO₄ and Duckweed water plant shows edema in the

submuscularis layer (black arrow) with minor adhesion between villi (Figure 9). The microscopic examination of the group treated with CuSO₄ and Azolla reveals enteritis with mucus secretion (Figure 10). The water plant in the group subjected to CuSO₄ and a mixture of Azolla and Duckweed showed mid enteritis involves hydropic degeneration in the gland's epithelial cells (Figure 11).



Figure 3: The gills of *C. carpio* subjected to CuSO₄ for 56 days display hyperplasia of mucus cells (black arrow) with edema (red arrow), sloughing of epithelial cells (green arrow), H&E, 40×9.1X.

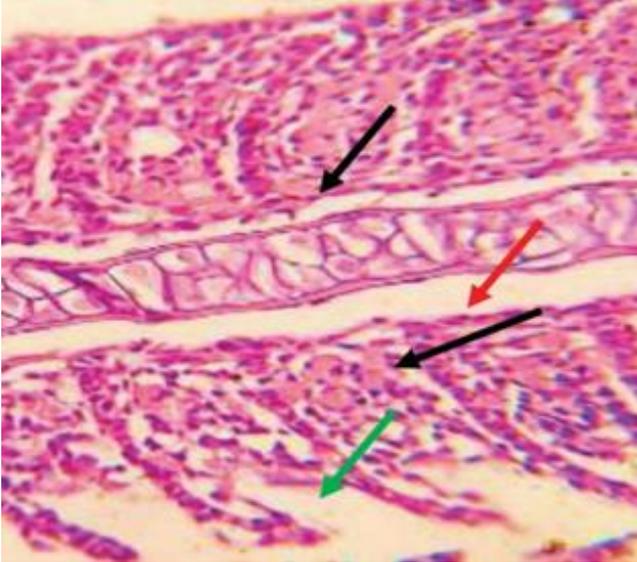


Figure 4: microscopic examination of gills in *C. carpio* exposed to CuSO₄ for 56-day show hemorrhage (black row) with hydropic degeneration of epithelial cells (red row). H&E, 10×5.1X.

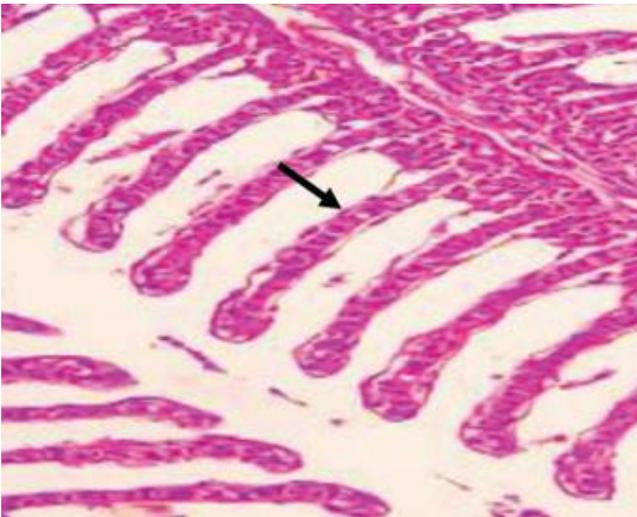


Figure 5: microscopic examination of gills in *C. carpio* exposed to CuSO₄ with Duckweed for 56 days show normal interlamellar space (black arrow) H&E, 10×7.5X.

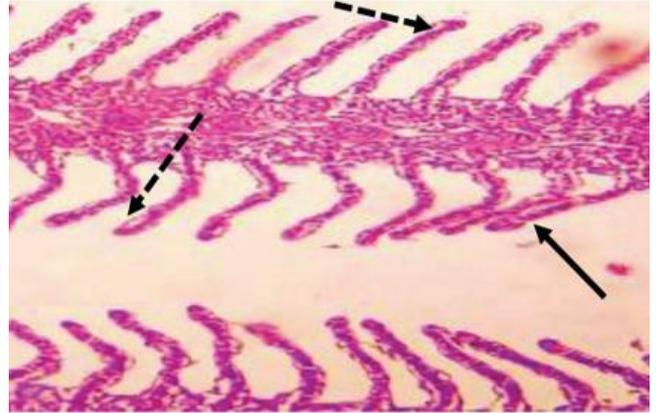


Figure 6: microscopic examination of gills in *C. carpio* exposed to CuSO₄ with Azolla and Duckweed for 56-day show loss straight secondary gill filaments (black arrow) with hydropic degeneration of epithelial cells (black dot arrow), H&E, 40 ×4.8X.

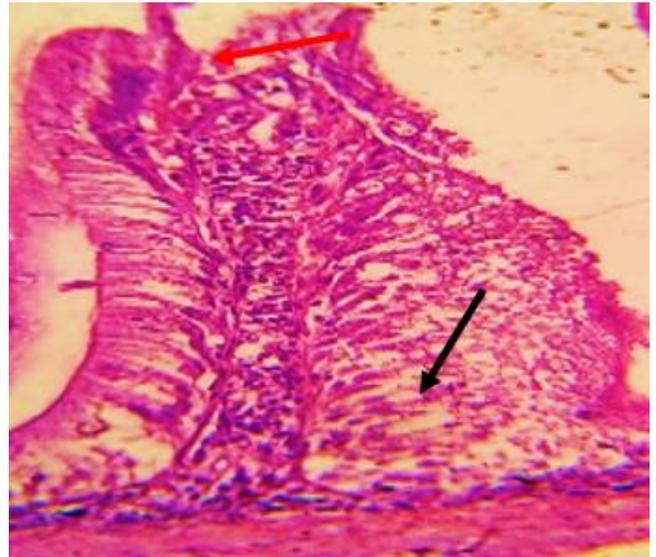


Figure 7: A microscopic analysis of the gut of *C. carpio* subjected to CuSO₄ for 56 days reveals severe hydropic degeneration. epithelial cells lining villi (black arrow) with damage and sloughing of the villi, apex (red arrow). H&E, 40×1.8X.

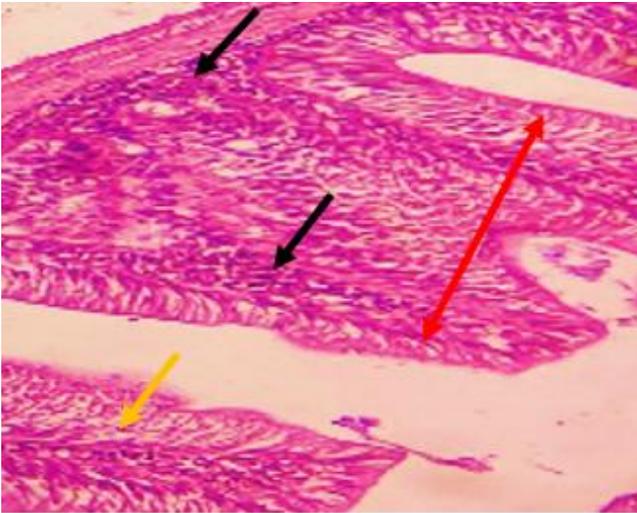


Figure 8: microscopic Photograph of intestine in *C. carpio* exposed to CuSO_4 for 56 days show inflammatory cell infiltration (black arrow) with villi attachment (red two head arrow), severe hydropic degeneration in epithelial cells (yellow arrow) H&E, 40×3.5X.

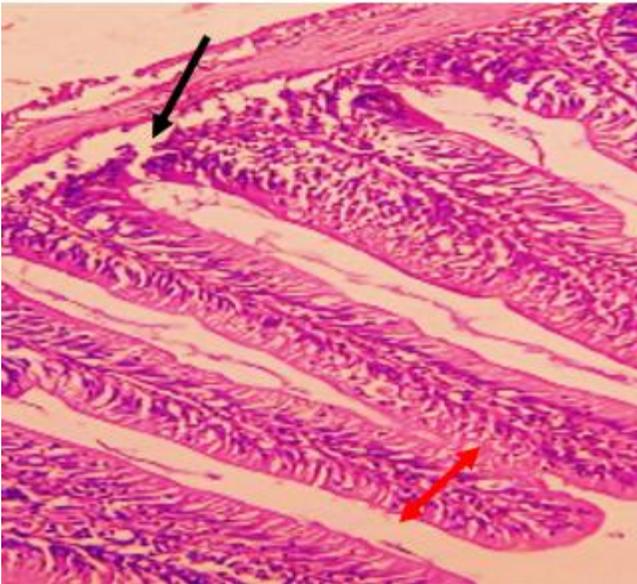


Figure 9: Photograph of intestine in *C. carpio* exposed to CuSO_4 with Duckweed for 56-day show edema in the submucosal layer (black arrow) with mild adhesion between villi (red arrow) H&E, 10×5.1X.

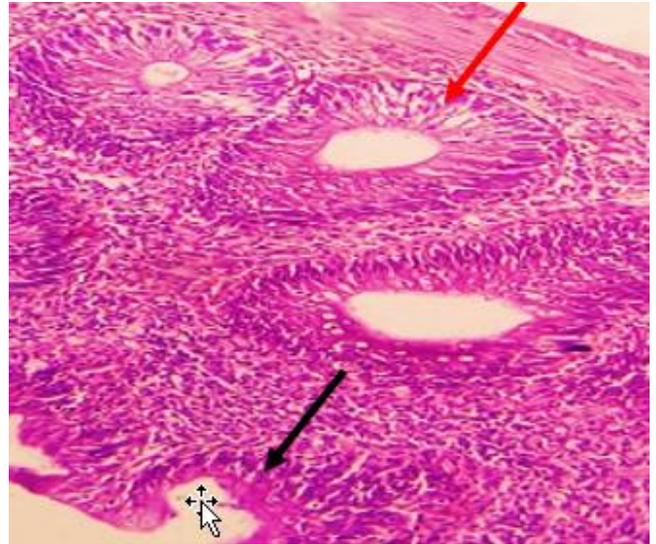


Figure 10: Photograph of intestine in *C. carpio* exposed to CuSO_4 with Azolla for 56 days show enteritis (black arrow) with mucus secretion (red arrow) H&E, 10×3.1X.

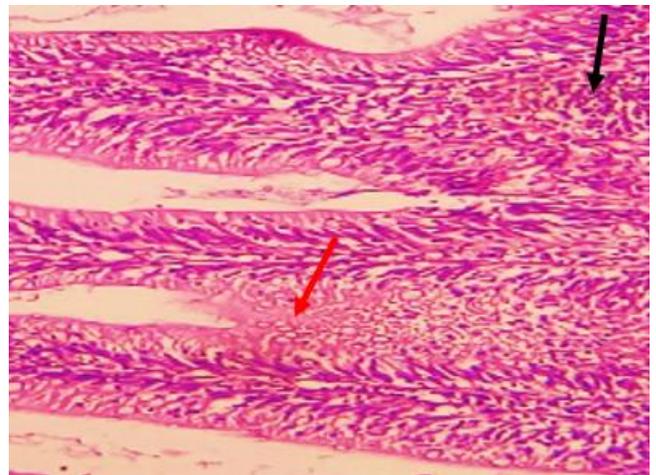


Figure 11: Photograph of intestine in *C. carpio* exposed to CuSO_4 with Azolla and Duckweed for 56 days show mid enteritis (black arrow) with hydropic-degeneration in the epithelial cells lining gland (red arrow) H&E, 40 ×4.3X.

Discussion

Fat is a crucial nutrient providing energy for life activities, and its fatty acid profile is critical in human and living organisms, as it can either protect against or induce coronary heart disease (28-30). Tang *et al.* (31) displayed that palmitic acid, linoleic acid and linolenic acid were the dominant components of fatty acid in Duckweed. Mary *et al.* (18), reported for fatty acids profile Duckweed, the PUFA and MUFA were significantly higher compared to the saturated fatty acids. Azolla contains significant levels of

fatty acids, including MUFA, PUFA and SF, this lipid fraction can be used to make high quality biodiesel, but this requires an additional fractionation stage and for the growth and proper performance of fish (32).

Recent research in nutrition and food science has emphasized the potential antioxidant, antimicrobial, and anti-inflammatory activities of plant products, including flavonoids and phenolic compounds, which are crucial natural compounds that can prevent coronary heart disease (33). A study by Ilhami *et al.* (34) reported that Duckweed found to be effective antioxidants due to high content of phenolic and flavonoids. Al-Khafaji *et al.* (29) pointed that Azolla includes several important phytochemicals such as flavonoids and phenols, these bioactive compounds contribute to therapeutic properties including anti-inflammatory, antioxidant, anti-hypertensive and antidiabetic, the presence of hydroxyl group in these compounds effective scavenging of free radicals. Many manufactured and natural chemicals dissolve in freshwater. Fish can be exposed to a wide range of pollutants. such as heavy metal salts such as CuSO₄, and have evolved to the effects of naturally occurring compounds in the water. Copper can accumulate in fish organs, causing redox reactions that produce free radicals, disrupting various physiological processes and altering morphology (35). Copper sulphate, a chemical copper, is utilized in industrial and agricultural applications. processes as a component of pesticide formulations. Numerous studies have demonstrated the effects of this chemical at varying dosages, including behavioral abnormalities,

decreased development, and reproductive issues (36,37). These findings are consistent with a study in which researchers concluded that copper sulfate might increase total cholesterol levels in mice (38,39). One study reported that tilapia fish in the experimental group (which received Azolla) had lower levels of total cholesterol compared to tilapia fish in the control group. This may be because Azolla contains compounds called sterols, which are similar in structure to cholesterol. Sterols can reduce the absorption of cholesterol from the intestine. In addition, Azolla is rich in fiber, which can help lower cholesterol levels by binding cholesterol in the intestines and removing it from the body (40,41). Copper may also cause physiological changes and protein dysfunction. Excessive copper exposure in fish can damage the gills, intestines, and sensory system (42,43). Oslo Azolla concede as Metal- cleansed water. It was revealed that metals (iron and copper) can be removed by *A. pinnata* and *L. minor* (Duckweed) (44,45). They concluded that mildly polluted wastewater may be cleaned by running it through ponds holding one or both of these water plants. Saxena discovered that a 2:1 blend of Lemna and Azolla may adequately filter substantially contaminated manufacturing wastewater for agricultural application (46,47).

Conclusion

The study substantiated the nutritional value of Duckweed and Azolla meal in the diets of *C. carpio* communis young. It appears to be an acceptable substitute for fishmeal in world diets because it has no negative effects on the histopathology or blood biochemical markers of *C. carpio* communis. With its intensification, Azolla and Duckweed meal might be successfully introduced to provide inexpensive and environmentally acceptable diets for raising large quantities of *C. carpio* communis fingerlings.

Conflict of interest

None.

Acknowledgments

The thanks extended to College of Agriculture and Forestry, University of Mosul, to support current study.

Reference

1. Xiong B, Xu T, Li R, Johnson D, Ren D, Liu H, Xi Y, Huang Y. Heavy metal accumulation and health risk assessment of crayfish collected from cultivated and uncultivated ponds in the Middle Reach of Yangtze River. *Sci Total Environ.* 2020;739:139963. DOI: [10.1016/j.scitotenv.2020.139963](https://doi.org/10.1016/j.scitotenv.2020.139963)
2. Ghosh A, Kaviraj A, Nielsen IE. A comparative evaluation of the effects of copper and nickel on the growth of the freshwater fish *Cyprinus carpio* and amelioration by *Pistia stratiotes*. *Toxicol Environ Health Sci.* 2021;13:363–74. DOI: [10.1007/s13530-021-00097-3](https://doi.org/10.1007/s13530-021-00097-3)
3. Abubakar MY, Ahmad KB, Shamsuddin R, Muhammad HM, Haladu M, Adam AB. Heavy metal pollution in aquatic ecosystems: a review of toxic impacts and remediation strategies. *J Sci Artif Intell.* 2024;1(1):75–86. DOI: [10.58578/AMJSAL.v1i1.3387](https://doi.org/10.58578/AMJSAL.v1i1.3387)
4. Al-Sarraj ES, Eskandera MZ, Al-Tae SK. Heavy metal pollution in Iraqi rivers and impact on human and fish health: a review. *Biol Appl Environ Res.* 2022;6(2):95–112. DOI: [10.51304/baer.2022.6.95](https://doi.org/10.51304/baer.2022.6.95)
5. El-Gendy MA, El-Khodary AM, El-Aziz HA, Abdel-Aziz MM. Assessment of copper contamination in groundwater and surface water from agricultural land: a review. *Sci Total Environ.* 2021;774:145860. DOI: [10.1016/j.scitotenv.2021.145860](https://doi.org/10.1016/j.scitotenv.2021.145860)
6. Al-Kattan HA, Al-Bassam NH. Evaluation of adding sweet bean (*Foeniculum vulgare*) and roselle (*Hibiscus sabdariffa*) seed on enhancing growth and performance of common carp *Cyprinus carpio*. *Mesopotamia J Agric.* 2024;52(2):58–67. DOI: [10.33899/mja.2024.146824.1374](https://doi.org/10.33899/mja.2024.146824.1374)
7. El-Khodary AM, El-Gendy MA, El-Aziz HA, Abdel-Aziz MM. Concentrations of copper in groundwater and surface water in the vicinity of agricultural land in the Nile Delta, Egypt. *J Environ Sci Health A Tox Hazard Subst Environ Eng.* 2022;57(9):1068–77. DOI: [10.1080/10934529.2022.2037168](https://doi.org/10.1080/10934529.2022.2037168)
8. Parks AN, Cantwell MG, Katz DR, Cashman MA, Luxton TP, Clar JG, Perron MM, Portis L, Ho KT, Burgess RM. Assessing the release of copper from nanocopper-treated and conventional copper-treated lumber into marine waters II: forms and bioavailability. *Environ Toxicol Chem.* 2018;37(7):1969–79. DOI: [10.1002/etc.4128](https://doi.org/10.1002/etc.4128)
9. Al-Sheikh AA, Al-Dabbagh MA, Al-Dabbagh S. Efficacy of copper sulfate as a fish parasite control agent: a review. *Aquaculture.* 2022;538:737107. DOI: [10.1016/j.aquaculture.2022.737107](https://doi.org/10.1016/j.aquaculture.2022.737107)
10. Manne R, Kumaradoss MM, Iska RS, Devarajan A, Mekala N. Water quality and risk assessment of copper in drinking water stored in copper

- container. Appl Water Sci. 2022;12:27. DOI: [10.1007/s13201-021-01542-x](https://doi.org/10.1007/s13201-021-01542-x)
11. Lasiene K, Straukas D, Vitkus A, Juodziukyniene N. The influence of copper sulphate pentahydrate (CuSO₄·5H₂O) on the embryo development in the guppies (*Poecilia reticulata*). Ital J Anim Sci. 2016;15(3):529–35. DOI: [10.1080/1828051X.2016.1188226](https://doi.org/10.1080/1828051X.2016.1188226)
 12. Ahmed SA, Lodhi S, Shukla S. Copper toxicity in aquatic ecosystem: a review. Int J Fish Aquat Stud. 2023;11(4):134–8. DOI: [10.22271/fish.2023.v11.i4b.2835](https://doi.org/10.22271/fish.2023.v11.i4b.2835)
 13. Al-Sheikh MM, Al-Sheikh AA, Al-Dabbagh S. The effects of copper sulfate treatment on the growth and development of fish: a review. Aquaculture. 2022;538:737108. DOI: [10.1016/j.aquaculture.2022.737108](https://doi.org/10.1016/j.aquaculture.2022.737108)
 14. Ngoc TV, Thanh TT, Nhung NT, Tuyet NTT. Accumulation of copper in catfish (*Clarias gariepinus*) cultured in a copper-contaminated pond in Vietnam. Environ Sci Pollut Res. 2022;39(15):12767–78. DOI: [10.1007/s11356-022-16429-y](https://doi.org/10.1007/s11356-022-16429-y)
 15. Al-Ni'ma BA, Al-Mandee FA. New occurrence of two exotic hydrophytes *Azolla filiculodes* Lam. and *Hydrilla verticillata* (L.f.) Royle in Tigris River within Mosul city. Mesopotamia J Agric. 2023;51(3):46–55. DOI: [10.33899/mja.2023.142415.1284](https://doi.org/10.33899/mja.2023.142415.1284)
 16. Mosha SS. A review on significance of Azolla meal as a protein plant source in finfish culture. J Aquac Res Dev. 2018;9(7):1–7. DOI: [10.4172/2155-9546.1000544](https://doi.org/10.4172/2155-9546.1000544)
 17. Kaakarlu SV, Kuppasamy A. A comprehensive study on the assessment of chemically modified *Azolla pinnata* as a potential cadmium sequestering agent. Int J Exp Res Rev. 2023;36:1–19. DOI: [10.52756/ijerr.2023.v36.001](https://doi.org/10.52756/ijerr.2023.v36.001)
 18. Mary O, Kevin M, Kevin O, Paul O, Patricia M. Nutritional composition of Duckweed (*Lemna minor*) cultured with inorganic fertilizer and organic manure in earthen ponds. J Limn Fresh Fish Res. 2023;9(3):123–129. [\[available at\]](#)
 19. Al-Tae NT, Mohammad MA, Abdul-Majeed AF. The effect of partial replacement of animal protein with Duckweed grown in the treatment unit of Nineveh pharmaceutical factory water on the growth performance of common carp *Cyprinus carpio*. Mesopotamia J Agric. 2024;52(3):22–36. DOI: [10.33899/mja.2024.145298.1316](https://doi.org/10.33899/mja.2024.145298.1316)
 20. Bojarski B, Witeska M, Kondera E. Blood biochemical biomarkers in fish toxicology – a review. Animals. 2025;15(7):965. DOI: [10.3390/ani15070965](https://doi.org/10.3390/ani15070965)
 21. Ramesh K, Manikandan V, Sivasubramanian S, Sivakumar R. Blood parameters as bioindicators of environmental pollution in fish. Environ Sci Pollut Res. 2022;39(14):12054–77. DOI: [10.1007/s11356-022-17929-5](https://doi.org/10.1007/s11356-022-17929-5)
 22. El-Ghandour MA, Al-Sheikh MM, Al-Dabbagh S. Use of blood parameters as biomarkers. Sci Total Environ. 2023;812:154313. DOI: [10.1016/j.scitotenv.2022.154313](https://doi.org/10.1016/j.scitotenv.2022.154313)
 23. Al-Sheikh MM, Al-Sheikh AA, Al-Dabbagh S. Histopathological effects of copper and zinc on the kidneys of Nile tilapia (*Oreochromis niloticus*). Environ Sci Pollut Res. 2022;39(14):12054–77. DOI: [10.1007/s11356-022-17929-5](https://doi.org/10.1007/s11356-022-17929-5)
 24. Li H, Zhang Y, Xu X, Li S. Effects of zinc on the histopathology of the kidneys of rainbow trout (*Oncorhynchus mykiss*). Ecotoxicol Environ Saf. 2022;191:112046. DOI: [10.1016/j.ecoenv.2022.112046](https://doi.org/10.1016/j.ecoenv.2022.112046)
 25. Roshidi MH, Mahyuddin HS, Mohamad MN, Noh AL. The effect of different fertilizer and extraction method on secondary metabolites of *Azolla pinnata*. Acta Chem Malays. 2020;4(1):28–32. DOI: [10.26480/acmy.01.2020.28.32](https://doi.org/10.26480/acmy.01.2020.28.32)
 26. Altaee RT. Preparation of silver nanoparticles of apigenin and catechin plant extracts and their antioxidants effect in experimental animals [Ph.D. dissertation]. Iraq: University of Mosul, College of Education for Pure Sciences; 2020.
 27. Chakrabarti R, Clark WD, Sharma J, Goswami RK, Shrivastav AK, Tocher DR. Mass production of *Lemna minor* and its amino acid and fatty acid profiles. Front Chem. 2018;6:479. DOI: [10.3389/fchem.2018.00479](https://doi.org/10.3389/fchem.2018.00479)
 28. Jingwen XU, Yanting S, Yi Z, Gordon S, Xiuzhi SS, et al. Duckweed (Lemnaceae) for potential human nutrition: a review. Food Rev Int. 2021;37(8):1–21. DOI: [10.1080/87559129.2021.2012800](https://doi.org/10.1080/87559129.2021.2012800)
 29. Al-Khafaji HH, Altameme HJ, Alsharifi SM. Detection of bioactive compounds in the methanolic extract of *Azolla filiculoides* Lam. fern by GC-MS technique. Iraqi J Agric Sci. 2022;53(4):922–32. DOI: [10.36103/ijas.v53i4.1714](https://doi.org/10.36103/ijas.v53i4.1714)
 30. Paul B, Adrie VH, Henriette S, Gert-Jan R, Klaas G. Lipid yield and composition of *Azolla filiculoides* and the implications for biodiesel production. Bioenerg Res. 2016;9:369–77. DOI: [10.1007/s12155-015-9697-9](https://doi.org/10.1007/s12155-015-9697-9)
 31. Tang J, Li Y, Ma J, Cheng JJ. Survey of Duckweed diversity in Lake Chao and total fatty acid, triacylglycerol profiles of representative strains. Plant Biol J. 2015;17:1066–72. DOI: [10.1111/plb.12347](https://doi.org/10.1111/plb.12347)
 32. Yin Y, Yu C, Yu L, Zhao J, Sun C, Ma Y, Zhou G. The influence of light intensity and photoperiod on Duckweed biomass and starch accumulation for bioethanol production. Biotechnol. 2015;187:84–90. DOI: [10.1016/j.biortech.2015.04.015](https://doi.org/10.1016/j.biortech.2015.04.015)
 33. Mukherjee AK, Kalita P, Unni BG, Wann SB, Saikia D, Mukhopadhyay PK. Fatty acid composition of four potential aquatic weeds and their possible use as fish-feed nutraceuticals. Food Chem. 2010;123:1252–4. DOI: [10.1016/j.foodchem.2010.05.057](https://doi.org/10.1016/j.foodchem.2010.05.057)
 34. Ilhami G, Ekrem K, Ebru A, Fevzi T, Olcay H. Antioxidant, antibacterial and anticandidal activities of an aquatic plant: Duckweed (*Lemna minor* L., Lemnaceae). Turk J Biol. 2010;34:175–88. DOI: [10.3906/biy-0806-7](https://doi.org/10.3906/biy-0806-7)
 35. Lin L, Liu J, Qin Q. Neurodevelopmental effects of prenatal exposure to pesticides: a systematic review. Environ Health Public Health. 2022;13(4):101–21. DOI: [10.1016/j.envh.2022.03.018](https://doi.org/10.1016/j.envh.2022.03.018)
 36. Di Martino G, Prisco A. Developmental effects of pesticides in animals and humans: a recent review. Int J Dev Biol. 2022;13(1):23–40. DOI: [10.1016/j.bidev.2022.01.002](https://doi.org/10.1016/j.bidev.2022.01.002)
 37. Lu Q, Zhao L, Liu J. Developmental effects of pesticides on animals and humans: an overview of the literature. Environ Dev. 2021;12(6):201–21. DOI: [10.1016/j.envdev.2021.06.022](https://doi.org/10.1016/j.envdev.2021.06.022)
 38. Zidani M, Hassan I, Abdo A. Effect of copper sulfate on total cholesterol levels in rats. Vet Sci. 2023;12(4):200–5. DOI: [10.3390/vetsci12040200](https://doi.org/10.3390/vetsci12040200)
 39. Magouz FI, Dawood MA, Salem MF, Mohamed AA. The effects of fish feed supplemented with *Azolla* meal on the growth performance, digestive enzyme activity, and health condition of genetically improved farmed tilapia (*Oreochromis niloticus*). Ann Anim Sci. 2020;20(3):1029–45. DOI: [10.2478/aoas-2020-0003](https://doi.org/10.2478/aoas-2020-0003)
 40. Scoups DM. Copper toxicity in fish: a review of recent advances in our understanding. Fish Physiol Toxicol. 2023;43(1):1–19. DOI: [10.1007/s10695-022-01136-9](https://doi.org/10.1007/s10695-022-01136-9)
 41. Abdulla AG, Al-Ani EA, Al-Jubouri AA. Removal of heavy metals from contaminated water using floating aquatic plants: a review. Environ Sci Pollut Res. 2023;30(23):23552–66. DOI: [10.1007/s11356-023-14749-8](https://doi.org/10.1007/s11356-023-14749-8)
 42. Saxena D, Kumar S, Chauhan R. Treatment of industrial wastewater using a mixed culture of *Lemna minor* and *Azolla pinnata*. Int J Environ Sci Technol. 2022;19(8):5993–6006. DOI: [10.1007/s13762-022-02148-0](https://doi.org/10.1007/s13762-022-02148-0)
 43. Wang Y, Wang Y, Liu Y. Effects of lead exposure on nitrogen and phosphorus uptake by *Lemna minor*. Environ Sci Technol. 2023;57(1):201–8. DOI: [10.1021/acs.est.1c04090](https://doi.org/10.1021/acs.est.1c04090)
 44. Li S, Zhang J, Zhang C. Cadmium exposure reduces phosphorus uptake in *Azolla pinnata*. Chemosphere. 2022;293:129755. DOI: [10.1016/j.chemosphere.2022.129755](https://doi.org/10.1016/j.chemosphere.2022.129755)
 45. Saxena D, Kumar S, Chauhan R. *Azolla pinnata*: a promising plant for the bioremediation of heavy metal-contaminated wastewater. Int J Environ Sci Technol. 2022;19(8):5993–6006. DOI: [10.1007/s13762-022-02148-0](https://doi.org/10.1007/s13762-022-02148-0)
 46. El Naggar S, El-Mesery HS. *Azolla pinnata* as unconventional feeds for ruminant feeding. Bull Natl Res Cent. 2022;46:66. DOI: [10.1186/s42269-022-00752-w](https://doi.org/10.1186/s42269-022-00752-w)
 47. El-Gendy MA, El-Khodary AM, El-Aziz HA, Abdel-Aziz MM. Effect of *Azolla pinnata* as a feed supplement on the growth performance and lipid metabolism of broilers. Poult Sci. 2022;101(10):4272–83. DOI: [10.3382/ps/abz774](https://doi.org/10.3382/ps/abz774)

مجموعة من ثلاث مكررات وعلى مدى ٥٦ يوماً. المجموعة الأولى اعتبرت سيطرة سالبة (بدون معاملة)، والمجموعة الثانية السيطرة الموجبة عوملت مع كبريتات النحاس بتركيز ٠,٥ ملغم / لتر، المجموعة الثالثة هي مجموعة الأزولا وكبريتات النحاس والمجموعة الرابعة الطحلب البطي وكبريتات النحاس وأخيراً المجموعة الخامسة المجموعة بمزيج من الأزولا والطحلب البطي وكبريتات النحاس. بعد انتهاء وقت التجربة، تم جمع عينات الدم والأعضاء من الأسماك ثم تم تشريحها وإزالة الأعضاء. وقد سجلت اختلافات معنوية في مستويات الألبومين والبروتين الكلي والدهون الثلاثية والكوليسترول والكوليبيولين. كما تم تسجيل التغيرات المرضية في أنسجة خياشيم وأمعاء الأسماك، والتي أظهرت حدوث نزيف مع انحطاط مائي للخلايا الظهارية المعرضة لكبريتات النحاس. تظهر الأمعاء أيضاً حالات تنكس مائي شديدة في الخلايا الظهارية التي تغطي الزغابات، مع تدمير وتفسير قمة الخلايا. تسلط الدراسة الضوء على القيمة الغذائية لطحالب عدس الماء ووجبة الأزولا في النظام الغذائي للإصبيات، مما بثبت أنها بديل مقبول لمسحوق السمك وتوفر وجبات غذائية ميسورة التكلفة ومقبولة بيئياً للتربية على نطاق واسع. نستنتج من هذه الدراسة أن إضافة الأزولا وطحالب البط إلى غذاء الأسماك يؤثر بشكل كبير على التسمم بكبريتات النحاس.

التحليل النوعي، الخصائص المضادة للأوكسدة للأزولا والطحلب البطي في الأسماك المجهدة كيميائياً والمعرضة لكبريتات النحاس

فرح سمير صالح^١ و رنا طالب الطائي^٢

^١ فرع العلوم الأساسية، كلية الزراعة والغابات، ^٢ قسم الكيمياء، كلية التربية للعلوم الصرفة، جامعة الموصل، الموصل، العراق

الخلاصة

هدفت الدراسة الى تحديد التأثير المضاد للأوكسدة والوقائي المحتمل لنبات الأزولا، الطحلب البطي وخليطهما ضد التأثير السمي لكبريتات النحاس (٠,٥ ملغم /لتر) على أسماك الكارب. قسمت الدراسة إلى قسمين، الأول شمل استخلاص وتشخيص المركبات الفعالة من نبات الأزولا والطحلب البطي باستخدام تقنية الطور الغازي عالي الأداء وشمل القسم الثاني دراسة القدرة المضادة للأوكسدة لنبات الأزولا والطحلب البطي، تكونت التجربة من خمس مجاميع، تكونت كل