



# Using Immobilized Algae (*Scenedesmus quadricauda*) to Reduce Copper Element Toxicity in Common Carp Fish (*Cyprinus carpio*)

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Abbreviation: Nat. Env. & Poll. Technol.

Website: [www.neptjournal.com](http://www.neptjournal.com)

Received: 29-06-2024

Revised: 26-07-2024

Accepted: 07-08-2024

## Key Words:

Copper toxicity  
Common carp fish  
Immobilized algae  
Bioremediation  
LC50

## Citation for the Paper:

Ismaal, A., Salman, J. M. and Yass, M. J., 2025. Using immobilized algae (*Scenedesmus quadricauda*) to reduce copper element toxicity in common carp fish (*Cyprinus carpio*). *Nature Environment and Pollution Technology*, 24(1), D1694. <https://doi.org/10.46488/NEPT.2025.v24i01.D1694>

Note: From year 2025, the journal uses Article ID instead of page numbers in citation of the published articles.



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## ABSTRACT

The study assessed the efficiency of immobilized algae (*Scenedesmus quadricauda* (Turpin) Brébisson) in treating copper toxicity in common carp fish. Acute toxicity of copper towards carp fish was determined. Fish were exposed in aqueous tanks to different heavy metal concentrations (10, 15, 25, and 35 ppm) for 96 h to examine their response. The lethal concentration (LC50) of copper for common carp over 96 h was found to be 1.4 ppm, with fish mortality increasing gradually with higher metal concentrations. Subsequently, half of the LC50 concentration (0.7 ppm) was used as a chronic toxicity concentration, and fish were treated for 21 days to assess copper accumulation in their gills and muscles. Copper concentration in gills on day 5 of the experiment was  $16.89 \pm 2.2 \text{ mg.kg}^{-1}$  (Mean  $\pm$  S.D), a significant increase from in muscles, which recorded  $10.72 \pm 1.1 \text{ mg.kg}^{-1}$  (Mean  $\pm$  S.D). On day 21, the copper concentration decreased significantly in both gills ( $4.73 \pm 0.5 \text{ mg.kg}^{-1}$ ) and muscles ( $8.4 \pm 4.5 \text{ mg.kg}^{-1}$ ) compared to the control group (significant LSD 0.05). But the copper and algae group recorded on day 21 of the experiment (a significant decrease LSD 0.05) in both the gills ( $\text{mg.kg}^{-1}$  Mean  $\pm$  S.D) ( $4.73 \pm 0.5$ ) and the muscles ( $\text{mg.kg}^{-1}$  Mean  $\pm$  S.D) ( $8.4 \pm 4.5$ ) compared to the copper group. The removal rate in the gills was 75.57%, and in the muscles was 21.17%. Therefore, treatment with immobilized algae is an efficient and promising method for treating copper toxicity in aquatic environments.

## INTRODUCTION

Heavy metal pollution in water bodies is a major concern and has a serious impact on associated organisms, especially fish (Shahjahan et al. 2022). Heavy metals enter aquatic systems, dissolve in the water, and easily accumulate in various parts of aquatic organisms, including algae, bivalves, and fish, and then enter the contaminated fish's consumers (Cordoba-Tovar et al. 2022). Some HMs, including cobalt, copper, chromium, iron, manganese, molybdenum, nickel, selenium, and zinc, are toxic to organisms (Ogbuene et al. 2024).

Heavy metals can damage the fish's nervous system, which negatively disrupts the fish's interaction with the surrounding environment (Jamil et al. 2023).

Copper is a bioactive trace metal in aquatic environments and an important micronutrient for many aquatic species. It is an essential micronutrient that plays an important role as a cofactor in critical enzyme reactions related to body processes essential for survival in both humans and animals (Stern et al. 2010). The presence of excessive amounts of copper in the fish diet reduced fish appetite, which negatively affected feed utilization and fish growth (Takasusuki et al. 2004). Moreover, copper toxicity not only led to malformation of the reproductive organs but also led to a significant decrease in the fertility, fertilization, and hatching rates of many fish species (Forouhar et al. 2020).

Copper accumulates in fish when they are exposed to copper in high concentrations. Fish typically consume copper for metabolic functions; However,

it becomes toxic if fish are exposed to a higher concentration for a longer period. The gills are the first organ to accumulate heavy metals at a level above the concentration that is considered toxic through absorption along the surface of the gills, gut wall, and muscles (Annabi et al. 2013). The Copper accumulation is then distributed and bioaccumulated in major organs of fish, including the liver, spleen, and kidneys, and is transmitted through the blood (Tao et al. 2002).

Although many physical and chemical methods are available to remove these toxic heavy metals, most of these techniques appear ineffective when metal concentrations are less than  $100 \text{ mg.L}^{-1}$  (Salman et al. 2022). Since many heavy metals are water-soluble and dissolve in contaminated water, it is difficult to separate them by applying physical methods (Ahluwalia & Goyal 2007). In this case, biological methods such as bioremediation can be an attractive solution to correct the natural state of the environment from heavy metal pollution. The bioremediation process is very effective in reducing the toxicity of heavy metals by converting them into less harmful forms with the help of certain organisms or their enzymes to reduce pollution. This is an environmentally friendly and cost-effective way to revitalize the polluted environment (Ma et al. 2016).

Many biological agents are used to remove heavy metals from the aquatic environment (Tarekegn et al. 2020). Although heavy metals are usually toxic, organisms have developed specific resistance mechanisms and complex intracellular pathways. To utilize and detoxify heavy metals (de Alencar et al. 2017).

Algae are used in biotechnology to remove heavy metals from polluted aquatic environments because it has exhibit high biosorption capacity, allowing them to remove toxic heavy metals such as cadmium, copper, and lead (Abbas et al. 2014, Cirillo et al. 2012). This degradation is attributed to their ability to photosynthesize and secrete oxygen (Salman et al. 2023a), which leads to the breakdown of toxic organic elements, including heavy metals (Kumar et al. 2021). The removal mechanisms are the use of the algal cell wall containing substances such as fucoidan and alginate that act as heavy metal binding sites (Park et al. 2016, Mantzorou et al. 2018). In addition, algae produce compounds such as metallothioneins and phytochelatins that help in safely converting and storing heavy metals (Perales-Vela et al. 2006). Algae also exhibit cellular defense mechanisms that include anti-free radical systems, which help them adapt to the toxicity of heavy metals such as copper, such as superoxide dismutase (SOD) and catalase (CAT) activity (Morelli & Scarano 2004).

To overcome this problem, the culture of immobilized algae has been proposed in which the algae are trapped

within a polymeric matrix that allows nutrient access, improves nutrient removal efficiency, resists toxins, and protects them from predators (de-Bashan & Bashan 2010). Natural polymers such as alginate are commonly used in these matrices due to their availability, non-toxicity, and allowing the light transmittance necessary for photosynthesis processes (Moreno-Garrido 2008).

Immobilized algae systems have been tested by numerous studies for their efficiency in removing heavy metals. Significant uptake of Co, Zn, and Mn has been reported for *Chlorella salina* cells bound in alginate beads (Garnham et al. 1992). In another study, alginate beads of restricted algae *S. quadricauda* were used to monitor water quality in fish (tilapia) farming (Chen (2001); also, many studies deal with study the role of immobilized algae in removing pharmaceutical waste and evaluate to reduce toxicity by these algae (Obaid et al. 2024b, Obaid et al. 2023, Salman et al. 2022).

This study aims to evaluate the toxicological properties of common carp fish after exposure to copper, evaluate the extent of copper bioaccumulation in the gills and muscles of fish, and the ability of immobilized algae (*S. quadricauda*) to reduce copper toxicity in fish.

## MATERIALS AND METHODS

### Collecting and Acclimating Fish Samples

The fish used in this study, common carp (*Cyprinus carpio* Linnaeus), was obtained from a fish farm. The primary types of nets used for fishing are drift nets, surrounding nets, and trap nets on Al-Mahawil regain from Babil Governorate, middle of Iraq. The samples were transferred using plastic containers to the Advanced Environmental Laboratory in the Department of Biology, College of Science. The experiment was conducted in December 2023 and February 2024. Healthy fish were selected, and abnormal fish were excluded. The fish were acclimated to laboratory conditions for 21 days in aerated dechlorinated freshwater, changed every 48 h, and were fed twice daily with commercial fish food (Mostakim et al. 2015).

The fish samples were placed in an oval-shaped plastic aquarium with a capacity of 100 liters. Oxygen was maintained continuously for an average of 24 hours per day by aerating the containers. Chlorine-free water was used by storing tap water in tanks for 72 hours. The water temperature is adjusted using the air conditioner in the laboratory. Throughout the experiment, the water was changed every 48 hours.

### Preparation of Heavy Metal Solutions

To prepare 1000 mL of a  $0.1 \text{ mol.L}^{-1}$  solution of Copper(II)

nitrate, we have to dissolve 29.5646 g of  $\text{Cu}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  (100 % purity) in deionized or distilled water. After the solid is completely dissolved, dilute the solution to a final volume with deionized (distilled) water to prepare (10,15,20,25,35)  $\text{mg} \cdot \text{L}^{-1}$  from  $\text{Cu}(\text{NO}_3)_2$ .

### Preparation of Green Algal Media

Chu-10 modified growth media for green algae was used, and 2.5 mL of each stock solution was taken and supplemented to 1 L of distilled water, then sterilized using an autoclave, except for the stock solution ( $\text{K}_2\text{HPO}_4$ ), which was added at the end after sterilization to obtain 1 liter of Chu-10. The pH of Chu-10 was adjusted to 6.4 using 0.01(N) NaOH or HCl, and the medium was left until the next day for use in algal growth (Kasim et al. 1999).

### Preparation of Immobilized Algae

A 10 mL of algal cultured was placed in a beaker containing 100 mL of Chu-10 medium and cultured for 15 days under controlled conditions of  $286 \mu\text{E} \cdot \text{m}^2 \cdot \text{s}^{-1}$ , a light-dark period of 16:8 h, and a temperature of  $25 \pm 2^\circ\text{C}$  in a growth chamber (Chia et al. 2013). Then, 100 mL of cultured algae were placed in a beaker with 1000 mL of Chu-10 medium and incubated for 14 days (Tredici 2004). Each 50 mL algal sample taken in stationary phase at 12 and 14 days was centrifuged for 15 min at 3000 rpm to prepare immobilized algae with the following steps:

1. The concentrated algae were taken, an equal amount of sodium alginate solution (2%) was added, and it was shaken well to homogenize the ingredients.
2. Then this mixture (algae and alginates) is placed in a medical syringe or separating funnel. The mixture is dripped drop by drop into a beaker containing a 3% solution of calcium chloride with constant stirring. It is noted that the drops that come down from the syringe solidify in the calcium chloride solution, and then we use a tea strainer to remove the beads (frozen organisms) from the calcium chloride solution. It is then preserved in distilled water in a cold place (Adlercreutz & Mattiasson 1982, Al Mosawi et al. 2022).

### Water Quality

Water temperature, electrical conductivity, salinity, and pH were measured directly by a portable digital multimeter, Model 340i h SET, WTW, made in Germany after titration with standard solutions at (4.1, 7, 10.1) and a special buffer solution.

### Estimation of $\text{LC}_{50}$

Healthy and active fish were common carp (*Cyprinus*

*carpio*) ( $n = 72$ ), with total length and weight of fish of  $18.5 \pm 1.45$  cm and  $89.8 \pm 5.6$  g, respectively, and were kept in continuously aerated aquariums for laboratory acclimation. Conditions for 2 weeks under controlled natural lighting (12:12 h, light-dark).

The water in the containers was continuously oxygenated (air bubble drivers), and the water was replaced every 48 hours. The temperature was maintained at  $19 \pm 2^\circ\text{C}$ . For acclimation and experimental periods, commercial fish food was given to fish twice a day, but fish were deprived of food for 24 hours before the experiment and throughout the acute toxicity test (Mostakim et al. 2015).

The fish were exposed to different concentrations (10, 15, 20, 25, and 35  $\text{mg} \cdot \text{L}^{-1}$ ) of copper in the form of copper nitrate  $\text{Cu}(\text{NO}_3)_2$ .

To determine  $\text{LC}_{50}$  values for 96 h. Three aquaria were used for each concentration, each containing six fish in 70 L of dechlorinated tap water (Svoboda et al. 2001). One control group contained the same number of fish and the same volume of water but without Copper. Before planting the fish in the container, copper is added, and the water is aerated for an hour to distribute the element homogeneously in the water. In addition, the water in the container is replaced every 48 hours.

Mortality was recorded 24, 48, 72, and 96 h after exposure. Dead fish were removed to avoid possible deterioration in water quality (Gooley 2001). The lethal concentration ( $\text{LC}_{50}$ ) for 96 h was calculated using the probit method (Finney 1971). The water is changed every 48 hours, according to (Reish & Oshida 1987), who emphasized that the concentration in aquariums must be re-concentrated.

### Sub-Chronic Toxicity

Fish were exposed to 0.7 ppm Cu consecutively for 21 days as a sub-lethal concentration to estimate the chronic toxic effect using 35 experimental organisms.

### Removal Efficiency (R.E.)

$$R.E. \% = \frac{C1 - C2}{C1} \times 100$$

Where: R.E%: Removal efficiency, C1: Heavy metal concentration before treatment, C2: Heavy metal concentration after treatment (Al Mosawi et al. 2022).

### Estimation of Copper Residues in Fish

Heavy metals were determined in fish samples (collected, dried, and ground), where the fishmeal was digested by the acid digestion method (APHA 2017).

Where 3 g of the fish sample powder to be digested was placed inside a volumetric flask (25 mL), then (3 mL) of concentrated Perchloric acid solution ( $\text{HClO}_4$ ) was added to it. The beaker was covered using an hourglass bottle, and it was heated quietly on a hot plate, and we raised the temperature. Heat gradually until the digestion process is complete. When the mixture reaches the dryness stage, we leave the cup to cool and add again (3 mL) of concentrated nitric acid solution ( $\text{HNO}_3$ ). Cover the cup and continue heating until the digestion process ends, as obtain a mixture consisting of delicate colors, and a light one is called (a light-colored digester). Also, evaporate it until the dryness stage, and add 5 mL of hydrochloric acid (HCl) solution diluted with water in a ratio (1:1), then heat it so that the sample remaining after digestion dissolves, then add distilled water. The filtration process was carried out to eliminate remaining and insoluble substances, and the volume of the solution was adjusted according to the expected concentration in the samples to a volume of 100 mL, 50 mL, or less so that the sample is ready for analysis using an atomic absorption spectrophotometer (Shimadzu AA 7000).

### Statistical Analysis

This study used the analysis of variance (ANOVA), LSD, median, standard deviation, minimum, and maximum to find the significance among the study variances by using SPSS statistical program software (version 17).

## RESULTS

### Water Quality in the Aquarium

The water quality result had the following characteristics:

Table 1: The log of concentration and probit mortality for *C. carpio*.

Concentration $\text{mg.L}^{-1}$	Log of Concentration	Mortality rates %	Probit Mortality
0	0	0%	0
10	1	0	0
15	1.176091	20	4.16
25	1.39794	40	4.75
35	1.544068	60	5.25

pH  $8.5 \pm 0.3$ , temperature  $19 \pm 2^\circ\text{C}$ , dissolved oxygen  $6.4 \pm 0.45 \text{ mg.L}^{-1}$ , salinity  $1.2 \pm 0.1 \text{ ‰}$ , E.C.  $2360 \pm 48 \text{ }\mu\text{S.cm}^{-1}$ .

### Lethal Concentration 50 ( $\text{LC}_{50}$ )

The half-lethal concentration ( $\text{LC}_{50}$ ) of *C. carpio* was calculated within 96 h, and different concentrations of copper were used (10, 15, 25, and 35 ppm). It was observed that the rate of fish mortality increased with an increase in the copper concentration and duration of exposure. The concentration and probit mortality rate were recorded in Table 1.

The  $\text{LC}_{50}$  was determined by plotting a log graph of the concentration against the probate mortality of *C. carpio* exposed to copper (Fig. 1), and then the  $\text{LC}_{50}$  was calculated from the straight line equation and found to be 1.4 ppm. The fish were exposed for 21 days (chronic exposure) to a single concentration of 50% of the lethal dose (0.7 ppm).

### Estimation of Copper Residues in Fish

The concentration of copper in the gills and muscles of the control group, the copper group, the copper group, and the restricted algae is shown in Table 2 and Figs. 2 and 3. The concentration of copper in the gills was significantly higher

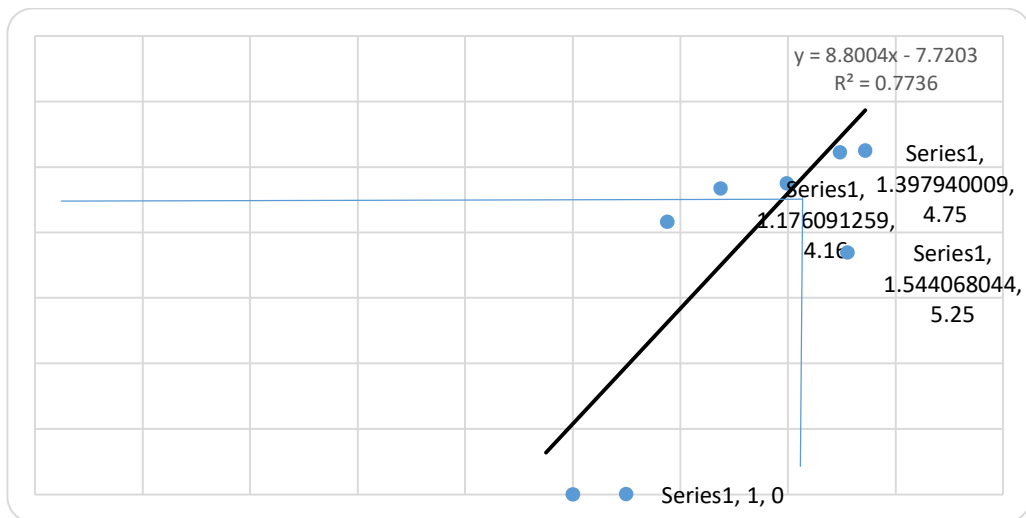


Fig. 1: Linear regression of toxicity in *C. carpio* exposed to copper ( $y = \text{probability}$ ,  $X = \text{concentration}$ ).

than in the muscles at different times after treatment in all groups in the experimental groups, and it was statistically significant (LSD 0.05).

The amount of copper remaining in the gills of the fish in the copper-treated group recorded a Mean  $\pm$ S.D. of  $16.89 \pm 2.2$  on the fifth day of the experiment, a significant increase (LSD 0.05) compared to the control group of  $1.45 \pm 0.2$  Mean  $\pm$ S.D. As for the twenty-first day of the experiment, the copper group recorded a Mean  $\pm$ S.D.  $8.11 \pm 1.9$  Significant increase (LSD 0.05) compared to the control group  $1.33 \pm 0.19$  Mean  $\pm$ S.D. The amount of residual copper in the gills of the fish of the copper and restricted algae group, Mean  $\pm$ S.D., was  $19.02 \pm 2.6$ . On the fifth day of the experiment, a significant increase was recorded (LSD 0.05) compared to the copper group, Mean  $\pm$ S.D.,  $16.89 \pm 2.2$ . On the twenty-first day of the experiment, the copper and algae group recorded a Restricted Mean  $\pm$ S.D  $4.73 \pm 0.5$  Significant decrease LSD 0.05) compared to the copper group Mean  $\pm$ S.D  $8.11 \pm 1.9$ .

The amount of copper remaining in the muscles of the fish in the copper-treated group recorded a Mean  $\pm$ S.D of  $10.72 \pm 1.1$  on the fifth day of the experiment, a significant increase (LSD) (0.05) compared to the control group ( $0.81 \pm 0.02$  Mean  $\pm$ S.D), while on the twenty-first day of the experiment the copper group recorded a Mean  $\pm$ S.D.  $6.33 \pm 1.8$  Significant increase (LSD 0.05) compared to the control group  $0.57 \pm 0.01$  Mean  $\pm$ S.D. The amount of copper remaining in the muscles of the fish of the copper and restricted algae group, Mean  $\pm$ S.D., was  $6.17 \pm 1.3$ . On the fifth day of the experiment, a significant decrease (LSD 0.05) was recorded compared to the copper group, Mean  $\pm$ S.D.,

Table 2: Copper residues ( $\text{mg.L}^{-1}$ ) in the gills and muscles of carp fish in the copper group, copper with immobilized algae group, and the control group.

Parameters	Treatment (A) Day (B)	Cu [ $\text{mg.kg}^{-1}$ ]	
		Gills Mean $\pm$ S.D	Muscles
Control	5	$1.45 \pm 0.2$	$0.81 \pm 0.02$
	21	$1.33 \pm 0.1$	$0.57 \pm 0.01$
Fish +Cu	5	$16.89 \pm 2.2$	$10.72 \pm 1.1$
	21	$8.11 \pm 1.9$	$6.33 \pm 1.8$
Fish +Cu+ Immobilized algae	5	$19.02 \pm 2.6$	$6.17 \pm 1.3$
	21	$4.73 \pm 0.5$	$8.45 \pm 1.7$
LSD(0.05) (A*B)		3.011	4.165

Table 3: Concentration of copper ( $\text{mg.L}^{-1}$ ) before & after treatment and removal efficiency of immobilized algae.

Treatment	Gills	Muscles
Cu group (day-5)	$16.89 \pm 2.2$ Mean $\pm$ S.D	$10.72 \pm 1.1$ Mean $\pm$ S.D
Cu + algae group (day-21)	$4.73 \pm 0.5$ Mean $\pm$ S.D	$8.45 \pm 1.7$ Mean $\pm$ S.D
Removal Efficiency%	75.57	21.17

$10.72 \pm 1.1$ . On the twenty-first day of the experiment, the copper and algae group recorded Restricted Mean  $\pm$ S.D  $8.45 \pm 1.7$  Non-significant increase compared with the copper group Mean  $\pm$ S.D  $6.33 \pm 1.8$ .

### DISCUSSION

Despite the importance of copper as a micronutrient in the metabolism of aquatic organisms, several studies have shown

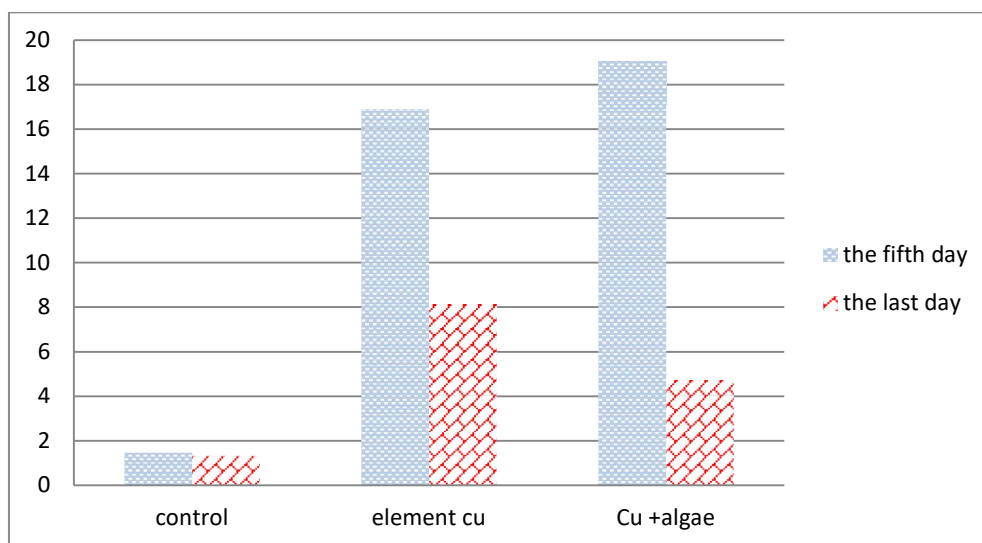


Fig. 2: Accumulation of copper (Cu) in the gills of common carp fish after exposure to copper.

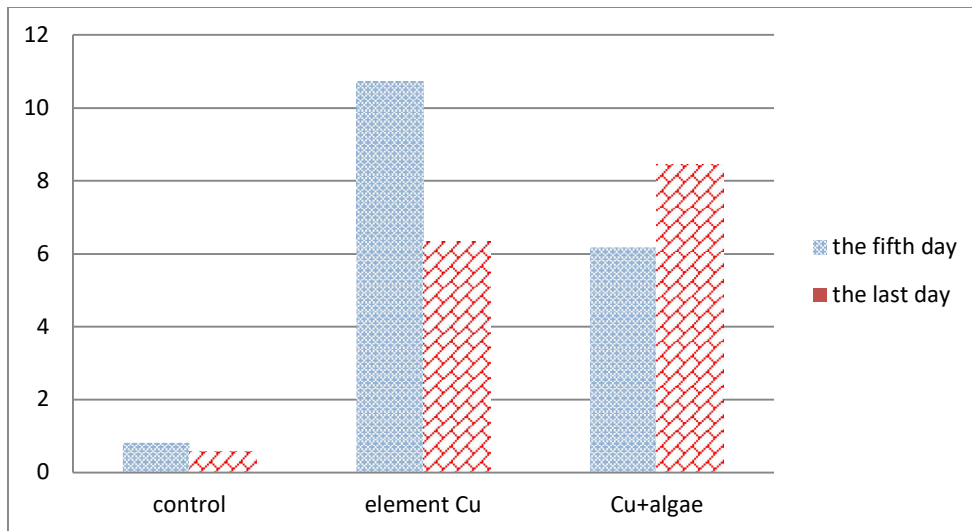


Fig. 3: Accumulation of copper (Cu) in the muscles of common carp fish after exposure to copper.

that even in very low concentrations, this mineral may affect fish survival (Farhangi & Jafaryan 2019). Human activities can release copper into the environment, especially into the land. Mining operations, along with incineration, are the main sources of copper release. Release into water occurs from the weathering of soil, industrial discharge, sewage treatment plants, and antifouling paints (Comber et al. 2022).

In the present study, the 96-hour LC<sub>50</sub> value of copper was found to be 1.4 ppm by the probit-phenylic analysis method to evaluate the data of the acute toxicity bioassay. Based on these results, it was found that there is a positive relationship between concentration levels and mortality rate. When the concentration level increased, the mortality rate also increased. The mortality rate was also related to the retention time of the copper in the water, as the longer the retention time of the copper in the water, the greater the mortality rate of the fish (Yustiati et al. 2024).

In a study by Goswami et al. (2021), carp fish were exposed to seven different concentrations of copper sulfate. It was found that the average lethal concentration (LC<sub>50</sub>) of copper in Amur carp fish for 96 h was 1.811. It was also noted that as the metal concentration increased, fish mortality also gradually increased. Other studies showed that (carp fish were exposed to seven different concentrations of copper. It was found that the average lethal concentration (LC<sub>50</sub>) of copper in *Cyprinus carpio* L. for 96 h was 2.65 mg.L<sup>-1</sup> (Al-Tamimi & Al-Azzawi 2015).

Mariyappan & Karuppasamy (2014) found that the mean LC<sub>50</sub> of copper for *Cyprinus carpio* L. was 38.36 mg.L<sup>-1</sup>. This variation in toxicity is due to several factors, such as the sensitivity of the test organisms, which is reflected in their

tolerance to toxic substances; The size of the organisms used in each bioassay; and the subjective factors of each individual (Farhangi & Jafaryan 2019).

Fish are naturally exposed to a variety of metals, including essential and non-essential elements. Copper is one of the essential minerals that, after being absorbed from the gills and intestines, is transported by metallothionein into the blood circulation, and some of it accumulates in various internal organs such as the liver, kidneys, and muscles (Peyghan et al. 2003). According to the results, the copper concentration in the gills in the 21st tom of the experiment was Mean  $\pm$ S.D 6.33 $\pm$ 1.8 in all groups. Since the gills are directly exposed to water and thus to toxic compounds, the percentage of pollutants in them is greater than that in the muscles (De Sousa et al. 1981). The present study results on the twenty-first day of the experiment are consistent with Kareem et al. (2022). Note that the concentration of some heavy metals in the muscles is 6.22 (1.45) (mg.kg<sup>-1</sup>) Mean (SD) and gills Mean (SD). 7.03 (0.95) (mg.kg<sup>-1</sup>) for common carp (*Cyprinus carpio*) from Dokan Lake, Sulaymaniyah, Iraq. As well as an approach to the results of Sobhanardakani & Jafari (2014), it was found that the copper concentration in the muscles of *Cyprinus carpio* fish h g was 4.0 $\pm$ 1.0 in Tahm Dam Lake, Iran.

The reason for the difference in results may be due to the size of the fish and the conditions of the study environment. A decrease in copper accumulation was observed on the twenty-first day of the experiment compared to the fifth day of the experiment in the gills and muscles, indicating that fish can reduce copper accumulation through several strategies, as fish increase the production of copper-binding proteins,

such as metallothionein, to reduce its toxic effect, as well as increase the production of copper-binding proteins. Copper excretion rates through the gills and kidneys for elimination (Kamunde et al. 2002). It increases the production of antioxidants such as glutathione to protect against oxidative copper damage (Monteiro et al. 2005).

According to US EPA standards, the maximum permissible concentration of lead is  $0.015 \text{ mg.L}^{-1}$  as it tends to have a high biological half-life thanks to its non-biodegradable nature, causing bioaccumulation and biomagnification in the food chain, which leads to serious environmental and health consequences (Obaid et al. 2024a). Many techniques are implemented to remove heavy metal ions, which are broadly classified as physical, chemical, and biological methods. Traditional physical and chemical methods are very expensive, inoperable at low concentrations of heavy metals below  $100 \text{ mg.L}^{-1}$ , and release hazardous derivatives solvents. In high quantities that are harmful to the environment, bioremediation is one of the most environmentally friendly and sustainable ways to reduce aquatic pollution, which plays an important role in improving the production of associated aquaculture systems (Jeyakumar et al. 2023).

Bioremediation of water contaminated with heavy metals using immobilized algae inside a polymeric matrix of alginate that allows the access of nutrients improves the efficiency of nutrient removal, resists toxins, and protects it from predators. These algae can be easily removed from the environment after treatment (Salman et al. 2023b), and therefore, it is an effective way to reduce the toxicity of heavy metals (de-Bashan & Bashan 2010).

In the current study, the group of copper and restricted algae, we observed an insignificant increase (LSD 0.05) in the concentration of copper in the gills on the fifth day of the experiment,  $19.02 \pm 2.6 \text{ Mean} \pm \text{S.D} (\text{mg.kg}^{-1})$  compared to the copper group,  $16.89 \pm 2.2 \text{ Mean} \pm \text{S.D} (\text{mg.kg}^{-1})$ .

The reason for this increase despite treatment with algae is the short treatment time (5 days). On the twenty-first day of the experiment, the concentration of residual copper in the gills of the fish of the copper group and the restricted algae was  $4.73 \pm 0.5 \text{ Mean} \pm \text{S.D. mg.kg}^{-1}$ . The treatment with algae led to There was a significant decrease in LSD (0.05) compared to the copper group  $8.11 \pm 1.9 \text{ Mean} \pm \text{S.D} (\text{mg.kg}^{-1})$ .

As for the muscles in the copper and algae-restricted group, we noticed a decrease in the copper concentration (LSD 0.05) on the fifth day of the experiment,  $6.17 \pm 1.3 \text{ Mean} \pm \text{S.D} (\text{mg.kg}^{-1})$ , compared to the copper group,  $10.72 \pm 1.1 \text{ Mean} \pm \text{S.D} (\text{mg.kg}^{-1})$ . This indicates the ability of algae to reduce the amount of copper in the water, which

leads to a reduction in the amount of copper in the muscles. On day 21 of the experiment, an insignificant increase in copper concentration was recorded in the copper and algae group,  $8.45 \pm 1.7 \text{ Mean} \pm \text{S.D} (\text{mg.kg}^{-1})$  compared to the copper group. This indicates that copper accumulation in the muscles is processed more slowly compared to the gills. The accumulation of heavy metals causes histological changes in fish muscles, providing an opportunity to assess fish health and information about potential health risks from their environment (Jaber et al. 2021). The use of natural products such as immobile algae in bioremediation is an effective way to reduce copper toxicity in aquatic environments.

A study has shown that fish can contract serious diseases such as blood poisoning when the fish are under stressful or unsuitable environmental conditions. This is considered the main cause of economic losses and public health impacts due to eating, handling, and transportation (Abaychi & Al-Saad 1988).

## CONCLUSION

The presence of copper in the water of fish farms at the half-lethal concentration LC50, although it did not cause deaths in the fish, may be lead to bioaccumulation of copper in the gills and muscles of the fish, thus causing potential risk to the fish and causing economic losses and impacts on the public health of those eating these fish in the event of contamination, but it was for biological treatment. Using immobilized algae has a significant effect on reducing the amount of copper through its high ability to absorb it from polluted water. Therefore, this technique is very suitable for controlling water quality and aquaculture.

## ACKNOWLEDGMENTS

The researcher extends his thanks and appreciation to the Department of Biology, College of Science at the University of Babylon, for their assistance in supporting this work.

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