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Acute Toxicity Effects of Ash-Polluted Diet on the Common Carp (*Cyprinus Carpio* L.1758)

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ABSTRACT

The current study aimed to determine the impact of sublethal concentrations of ash from the Durah Thermal Power Plant, mixed with polluted diet, on the biological aspects of *Cyprinus carpio*. The fish were exposed in the laboratory to diets containing 5, 10, 15, and 20 g/kg of ash. The LD50 values were 17.5, 11.71, 6.49, and 5.66 g/kg after 24, 48, 72, and 96 hours of exposure, respectively. The results showed a positive relationship between the percentage of mortality and the ash concentration in the diet, as well as the exposure duration. The percentage of deaths increased from 10 to 60% after 24 and 96 hours of exposure to a 5g/kg concentration. The LD100 was recorded at 15 and 20g/ kg, where 100% mortality occurred within 96 hours of exposure. It was concluded that the ash had a severe detrimental effect on the common carp, leading to environmental harm, especially when discharged directly in large quantities into water bodies.

INTRODUCTION

Thermal power plants are a major global concern due to their detrimental impact on ecosystems. Environmental pollution has become widespread, with pollutants increasing significantly since the onset of industrialization, particularly due to coal combustion for fossil fuel energy generation and subsequent gasification **(Quader *et al.*, 2020)**. The LD50 is a measure of the lethal dose of a substance that kills 50% of the test population, typically expressed in milligrams of substance per kilogram of body weight (mg/kg). The oral LD50 value for fish varies depending on the species and the substance being tested **(Rispin *et al.*, 2002)**. The Durah Power Plant (DPP), one of Baghdad's largest thermal power plants, is located in the southwestern part of the city. It uses crude oil to produce electricity, resulting in the generation of large amounts of fly ash, which is then discharged into the surrounding land near the Tigris River without treatment **(Nashaat *et al.*, 2019a)**. Ash particles are non-biodegradable, and once released into water bodies, they can be absorbed and adsorbed onto sediment particles or bioaccumulated in aquatic organisms **(Wang i., 2012; Al-Azzawi *et al.*, 2018)**. The toxic metals

in the ash cause various diseases in both micro- and macro-organisms within the food chain. These metals persist for long durations, have bio-magnification potential, and lead to degradation of both human and aquatic health **(Al-Naymi, 2019; Muftin *et al.*, 2020; Nashaat *et al.*, 2021; Alwan & Saeed, 2024)**.

Fish consumption by humans has become a major pathway for toxicant exposure, posing serious risks to human health. These risks include carcinogens, mutagens, damage to the nervous system, and adverse effects on the blood, liver, kidneys, and lungs **(IARC, 2009; Nashaat *et al.*, 2019b; Younis & Saeed, 2023)**. Additionally, exposure can lead to necrosis, irritation, poisoning, pathogens, and increased mortality rates in fish **(Al-Naymi *et al.*, 2019a)**. Fish in water bodies feed on live food, which has led to a number of regional studies focusing on the quantity and quality of fish live food, including works by **Al-Lami *et al.* (2004), Radi *et al.* (2005), Nashaat (2010), Nashaat *et al.* (2013), Hassan *et al.* (2014), Ala Allah *et al.* (2015), Nashaat *et al.* (2015, 2016), Rasheed *et al.* (2016), Abbas *et al.* (2017), Merhoon *et al.* (2017), Abed and Nashaat (2018), Al-Bahathy and Nashaat (2021), Nashaat *et al.* (2021), Majeed *et al.* (2021, 2022a,b), Abed *et al.* (2022), Al-Safi *et al.* (2022), Nashaat and Al-Bahathy (2022), Majeed *et al.* (2023), Idrees *et al.* (2024)** and **Nashaat and Salman (2024)**.

The common carp (*Cyprinus carpio*) is an important local fish species and is commonly used to study the toxic effects of metals. Therefore, the aim of the current study was to determine the impact of sublethal concentrations of a polluted diet containing ash from the Durah Power Plant (DPP) on the biological aspects of *Cyprinus carpio*.

MATERIALS AND METHODS

Fifty fish samples of the common carp *Cyprinus carpio* were divided equally into five groups as follows: four groups were fed on a polluted diet with ash at concentrations of 5, 10, 15, and 20g/ kg, while the fifth group served as the control.

All fish samples were collected from the Mussayib hatchery, with lengths ranging from 12 to 16 cm and a weight of 30 ± 4g. The fish were then transferred to the laboratory in plastic containers.

Only healthy fish were selected for the experiment. The fish were placed in aquaria containing 40L of aged aerated water (with 7.8mg/ L of dissolved oxygen) after a 12-day acclimatization period **(APHA, 2005)**. The aquaria were maintained under a photoperiod of 16 hours light and 8 hours dark, with water temperature ranging from 22 ± 4°C. The electrical conductivity was kept between 900-1200 µS/cm **(Falcón & Zohar, 2018)**.

The experimental diet was prepared as described in Table (1), and the fish were daily fed at a rate of 3% of their body weight.

Table 1. The fish diet components by **Al-Mawiahdi *et al.*(2022)**

Contents	Percentage%
Animal protein	10 %
Soy Beans	25 %
Yellow Corn	17 %
Black barley	22 %
Wheat Bran	25 %
Vitamins and Salts	1 %
Total	**100 %**

To prepare the wet food, all the ingredients were mixed, and a small amount of tap water was added. After placing the mixture into the chopping machine, ash was added at concentrations of 5, 10, 15, and 20 grams per kilogram of the fish diet, and the mixture was thoroughly combined. Ten fish of equal size from each exposure group and the control group were placed in duplicate treatment groups **(Yang & Chen, 2003)**.

The mixture was dried under the sun for 96 hours, with constant stirring to prevent fungal and parasite growth. The diet with 0% ash was provided to the control group, while all the exposure groups were fed twice daily at a rate of 3% of their body weight for four days **(Ruby *et al.*, 2013)**.

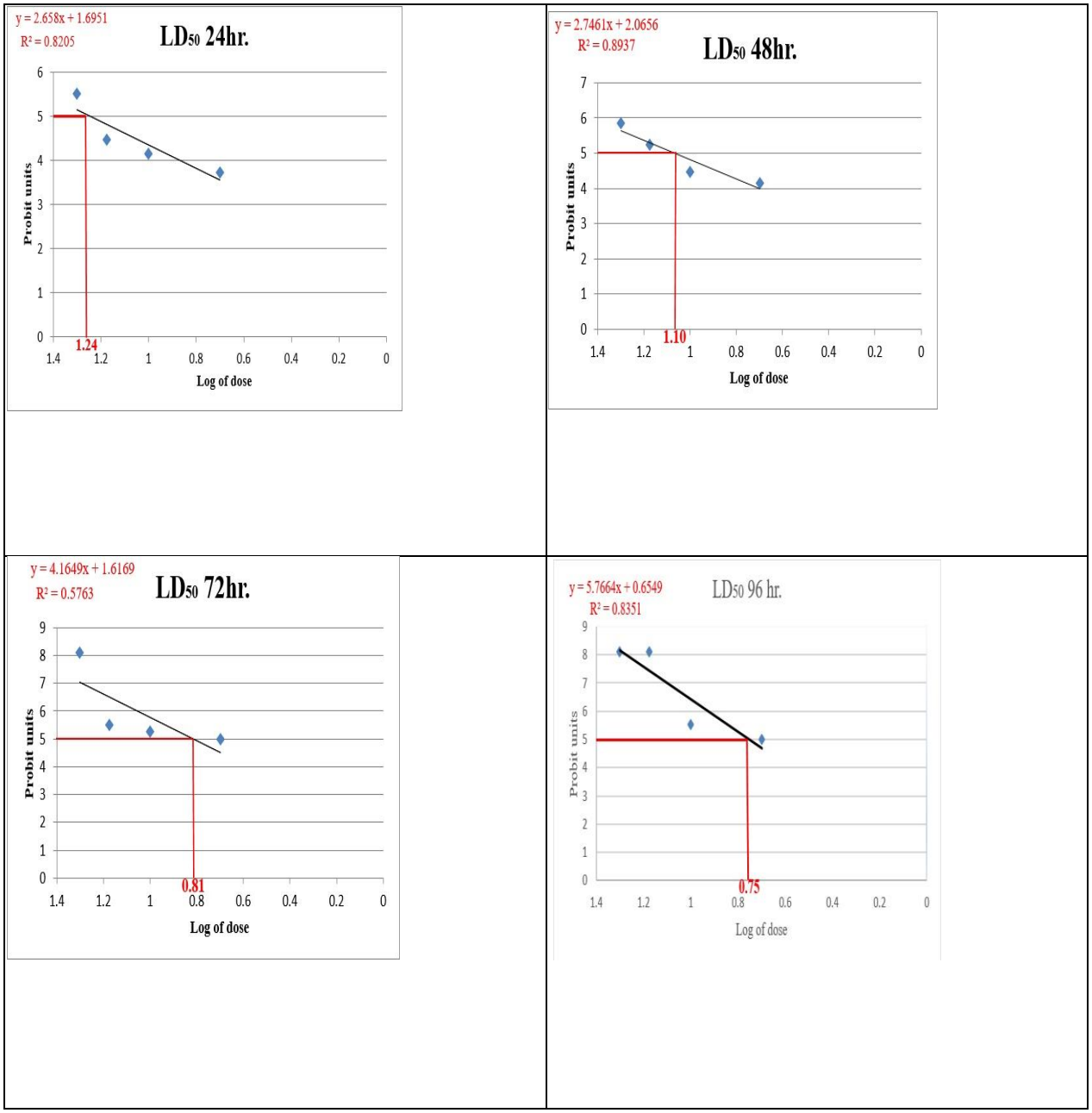
After each feeding, the remaining food was collected using an aquarium net to prevent depletion of dissolved oxygen and avoid microbial growth **(FAO, 1987)**.

All dead fish were retrieved during the exposure period, and mortality percentages were calculated after 24, 48, 72, and 96 hours **(Goldstein *et al.*, 1974; Buikema *et al.*, 1982; Goal, 2010)**.

The data were used to calculate the median lethal dose (LD50) for common carp using the Probit Analysis Statistical Method. The experiments were analyzed statistically using One-Way ANOVA and t-tests to calculate significant differences between the control and concentration groups at a probability level of $P \leq 0.05$. The results are expressed as Mean ± SE **(Finney, 1971; Goldstein, 1974; Buikema *et al.*, 1982)**.

RESULTS AND DISCUSSION

The LD50 values for a polluted diet with ash were 17.5, 11.71, 6.49, and 5.66g/ kg at 24, 48, 72, and 96 hours, respectively, as depicted in Figs. (1, 2, 3, and 4).



Figs. 1, 2, 3, 4. *Cyprinus carpio* median lethal dose after 24, 48, 72, and 96 hours of exposure

The survival rate of *C. carpio* significantly decreased over the exposure period, which may be associated to the species' high sensitivity to the chemical components of ash in the current investigation (Fig. 5).

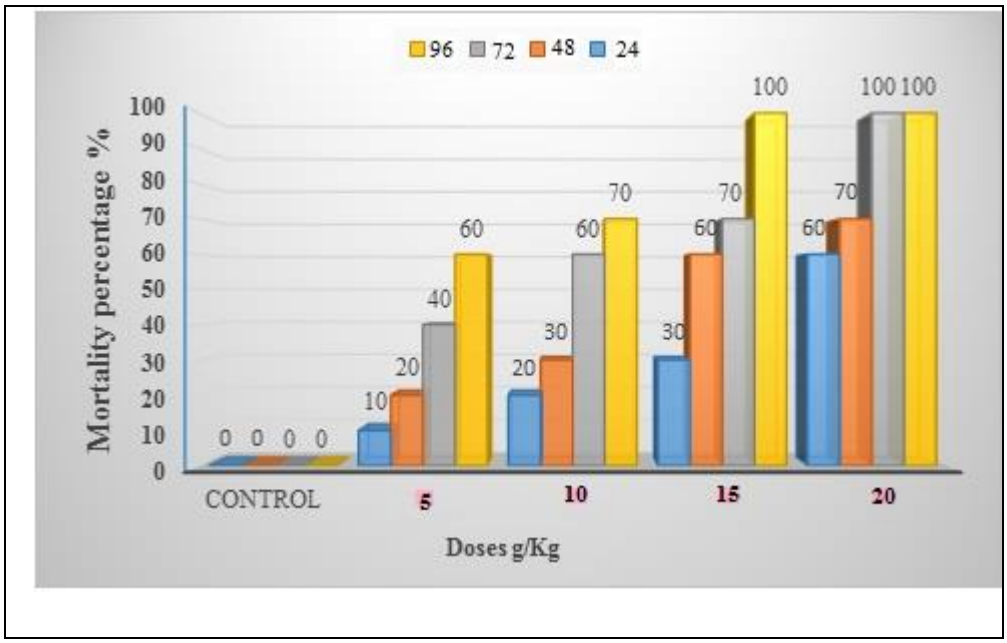


Fig 5. *Cyprinus carpio* mortality percentage during the exposure period

It was observed that the polluted diet with ash caused an inhibitory effect on the common carp over a long period. The highest mortality rate of 100% (LD100) was recorded when fish were fed a diet containing 20g/ kg of ash within 72 hours of exposure, while the lowest mortality rate of 10% was observed when the fish were fed a diet with 5g/ kg of ash within 24 hours. The mortality rate reached 60% after 96 hours of feeding with the same dose compared to the control group.

Before the fish died, several behavioral abnormalities were noted, including jerking movements, jumping, yawning, and coughing, especially when higher oral doses were consumed. The coughing was attributed to an increase in mucus production, which was deposited in the fish's gills. Dead fish were identified by counting the number of floating fish and by counting the fish at the bottom of the aquarium that showed no movement or external response to stimulation.

Sublethal toxicity research on fish and other aquatic organisms has become more common. The results of this study align with the findings of **Smet and Blust (2001)**, who observed complete mortality (100%) in the common carp after exposure to 20mg/ L of a similar substance. Likewise, **Sunmonu *et al.* (2009)** reported similar findings when they exposed *Heterobranchus bidorsalis* to varying concentrations of anthracene. Their results showed mortality rate increasing from 0 to 44.4% as the concentration increased from 0.25 to 1.25g/ L.

In a similar context, **Ibrahim *et al.* (2013)** noted that many fish samples from the Tigris River were polluted with heavy metals at levels exceeding the WHO's maximum permissible levels for fish. The current study’s findings are closely aligned with these

earlier studies, confirming the suitability of using such toxic compounds to expose aquatic organisms.

Additionally, **Xie *et al.* (2020)** found that fish cages in power plants accumulated high amounts of heavy metals. The study also found that fish in the Tigris River accumulated lead in their tissues at levels nearly four times higher than the maximum allowed **(Salman *et al.*, 2023)**. In a related study, fish fed diets polluted with 50mg/ kg cadmium recorded the lowest values of both body weight and weight gain. Serum total protein and albumin concentrations decreased significantly ($P< 0.001$), while liver enzymes SGOT and SGPT increased significantly ($P< 0.001$) as cadmium concentration in the fish diet increased **(Ayyat *et al.*, 2017)**. Similarly, a contaminated diet with *Aspergillus flavus* had significant adverse effects on the blood, body weight, and biochemical parameters of the common carp **(Mansoor *et al.*, 2018; Rasheed *et al.*, 2024)**.

The results of the present study are similar to those of **Al-Naymi (2019)**, who found LC50 values of 5.127, 2.506, and 1.619ppt for the common carp exposed to different concentrations of ash from a power plant for 24, 48, and 72 hours, respectively. When *C. carpio* was exposed to ash concentrations over the first 24 hours, the mortality rate increased to 100%. High mortality rates were also observed when cladocerans (*Simocephalus vetulus*), algae (*Chlorococcum humicola*), and *Pleuroxus hamulatus* were exposed to ash concentrations from the same power plant **(Al-Naymi *et al.*, 2019b, 2022, 2024)**.

The current findings underscore the importance of considering the health risks that toxicants pose to fish and other aquatic organisms in natural ecosystems. Heavy metals, in particular, are significant pollutants, as they impair metabolism, the circulatory system, and various physiological processes **(Yang & Chen, 2003; Majeed *et al.*, 2022)**. Ash-polluted fish diets cause harmful substances to bioaccumulate in the body and tissues, particularly at high concentrations **(Al-Musawi *et al.*, 2023)**. The polluted diet is absorbed through the alimentary canal and is transported to different organs. The liver is the primary target organ, where it causes hepatocyte damage and necrosis, ultimately leading to liver cell death and decreased liver function **(Cagauan *et al.*, 2004)**.

During ingestion, the diffusion of toxicants across the body organs decreases oxygen supply and causes reduced hyper-excitation, ultimately resulting in immediate fish death **(Ahmet, 2005)**.

CONCLUSION

Our research concluded that acute toxicity of polluted diets containing DPP ash had a severe detrimental effect on common carp fish and resulted in environmental harm, particularly when it was applied in large quantities and untreated directly to the Iraqi river. In addition, this result reveals that ash can cause an environmental damage aligned

with hematological and behavioral harmful effects on the aquatic organisms, especially when used at high concentration.

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