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Carotenoid-Rich Fungal Pigment from *Fusarium tricinctum* Mitigates Heavy Metal–Induced Toxicity in *Daphnia magna*

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Abstract
Heavy metals induce toxicity in aquatic organisms through bioaccumulation and oxidative stress. This study investigated the protective potential of a carotenoid-rich fungal pigment from *Fusarium tricinctum* against cadmium, zinc, chromium, mercury, and copper toxicity in *Daphnia magna*. HPLC and UV–Vis analyses identified β-carotene as the predominant carotenoid, while the pigment exhibited strong DPPH radical-scavenging activity. Acute toxicity assays determined 48-h LC_{50} values, and a concentration of 10 µg/L was selected for subsequent co-exposure experiments. ICP-MS analysis revealed that the fungal pigment significantly reduced tissue accumulation of all tested heavy metals compared with metal-only treatments. Moreover, pigment co-exposure attenuated oxidative stress by reducing malondialdehyde levels and modulating antioxidant responses, including SOD, CAT, and GSH. Overall, the F. tricinctum carotenoid pigment enhanced D. magna survival and alleviated heavy metal-induced oxidative damage, demonstrating its potential as a natural protective agent against metal stress in aquatic ecosystems.

Keywords Fungal pigment · *Daphnia magna* · Heavy metal toxicity · Carotenoids · Oxidative stress · Bioaccumulation

Introduction

Heavy metals are persistent and non-biodegradable pollutants of major concern in aquatic ecosystems because they can exert toxic effects even at low concentrations. They enter aquatic environments through industrial discharges, mining activities, agricultural runoff, and atmospheric deposition, where they are taken up by aquatic organisms and accumulate within their tissues. This accumulation disrupts essential biological processes, including survival, growth, reproduction, and oxidative balance, particularly in sensitive species such as Daphnia magna, which is widely recognized as an ecotoxicological model organism for assessing environmental contamination (Chupani et al. 2022; Paylar et al. 2024; Queiroz and Torresi 2025; El-Deeb Ghazy et al. 2024; Cojocaru et al. 2025). In recent years, considerable research attention has focused on natural pigments, particularly carotenoids of microbial and plant origin, as environmentally friendly bioactive compounds capable of alleviating oxidative stress associated with environmental pollutants, including heavy metals (Lim et al. 2023). These compounds have attracted increasing interest because of their remarkable antioxidant capacity and their ability to scavenge reactive oxygen species, thereby potentially reducing oxidative damage induced by heavy metal exposure (Lim et al. 2023; Medina 2015). Carotenoids are widely distributed in plants, algae, fungi, and photosynthetic microorganisms, where they function as photoprotective pigments, reactive oxygen species scavengers, and regulators of cellular redox homeostasis (Maoka 2020). In addition to their nutritional importance, carotenoids have been extensively investigated for their capacity to reduce lipid peroxidation, enhance antioxidant defense systems, and improve resistance to environmental stress in various biological models (Pereira da Costa and Miranda-Filho 2020; Magalhães et al. 2024). However, despite the growing interest in carotenoid-based bioactive compounds, the ecotoxicological potential of fungal carotenoids for alleviating heavy metal-induced toxicity in aquatic organisms remains largely unexplored (Maoka 2020; Siziya et al. 2022; Magalhães et al. 2024).

Among pigment-producing microorganisms, filamentous fungi represent an attractive yet comparatively

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underexplored source of natural carotenoids. In particular, species of the genus Fusarium synthesize carotenoid pigments that function as protective metabolites against adverse environmental conditions, including ultraviolet radiation, oxidative stress, heavy metal exposure, and elevated temperatures (Afroz Toma et al. 2023; Gulmez et al. 2023). These bioactive pigments contribute to cellular protection by scavenging reactive oxygen species, reducing lipid peroxidation, preserving membrane integrity, and maintaining cellular redox homeostasis (Liu et al. 2023; Zhuang et al. 2022). Beyond their antioxidant function, fungal carotenoids also participate in photoprotection, metabolic regulation, and stress adaptation, highlighting their potential as sustainable bioactive compounds for environmental applications (Heo et al. 2018; Patel et al. 2024; Zhuang et al. 2022).

Despite the increasing interest in natural carotenoids, information regarding the protective effects of fungal carotenoid pigments against heavy metal-induced toxicity in aquatic invertebrates remains scarce. Daphnia magna, owing to its short life cycle, high sensitivity to pollutants, and widespread use in standardized ecotoxicological assays, represents an excellent model organism for investigating the biological effects of environmental contaminants (Chupani et al. 2022; Paylar et al. 2024). Therefore, we hypothesized that the carotenoid-rich fungal pigment from F. tricinctum could attenuate heavy metal-induced oxidative damage and alter tissue metal accumulation, thereby improving survival in D. magna under acute metal stress conditions. To test this hypothesis, the present study aimed to evaluate the protective effects of carotenoid-rich fungal pigments produced by F. tricinctum on heavy metal-induced toxicity in D. magna by assessing survival, tissue metal accumulation, and oxidative stress biomarkers. The findings provide new insights into the potential of fungal carotenoids as sustainable natural bioactive compounds for alleviating heavy metal-induced oxidative stress in aquatic organisms.

Materials and Methods

Pigment Production and Analysis

Fusarium tricinctum OG2 (OQ975731) was cultivated in YPG medium under continuous illumination for 4 days. Carotenoids were extracted using hexane and petroleum ether according to the method described by Mohamed et al. (2020). The extracted pigments were qualitatively and quantitatively characterized by UV–Vis spectrophotometry and high-performance liquid chromatography (HPLC; Waters 2695, Waters Corp., Milford, MA, USA) equipped with a C18 reverse-phase column. Pigment identification was based on chromatographic retention times and characteristic absorption spectra. The antioxidant activity of the pigment extract was evaluated using the DPPH radical scavenging assay.

Chemical Preparation

Cadmium, mercury, zinc, copper, and chromium chloride salts (99.99% trace metal grade) were used in all experiments. Fresh stock solutions of each metal were prepared in ultrapure water (18.2 MΩ·cm) and stored in the dark at 4 °C until use. Working test solutions were prepared by diluting the stock solutions in ASTM hard water medium (ASTM A, 1980). All solutions were prepared using acid-washed glassware to minimize the risk of contamination.

Acute Toxicity Test and Determination of LC_{50}

Daphnia magna (<24 h old) were cultured according to OECD Test Guideline 202. Acute toxicity tests were conducted to determine the 48-h LC_{50} values of cadmium (Cd), mercury (Hg), chromium (Cr), zinc (Zn), copper (Cu), and the fungal pigment. Test concentrations were 5, 10, 20, 40, 60, 80, and 100 µg/L. For each concentration, ten individuals were placed in 250-mL glass beakers containing 200 mL of test solution, with three independent replicates per treatment. Control groups contained only reconstituted water. The organisms were not fed during the exposure period. After 24 and 48 h, immobilization was assessed, and individuals that were unable to swim within 15 s after gentle agitation of the beaker were considered immobile. The 48-h LC_{50} values were estimated by probit analysis using SPSS version 25.0 (IBM Corp., Armonk, NY, USA).

Exposure Experiment

Based on the immobilization responses observed across the tested concentration range and the calculated 48-h LC_{50} values, 10 µg/L was selected as a common exposure concentration for the subsequent experiments. This concentration was selected to provide a common exposure level across the tested metals and pigment while allowing comparative assessment of tissue metal accumulation and biochemical responses. The selected concentration was not intended to represent an equivalent toxicological threshold across the different metals, because the metals exhibited different acute toxicities. Cd, Hg, Cr, Zn, and Cu were therefore each applied at 10 µg/L. The fungal pigment was also applied at 10 µg/L. Independent exposure experiments were established for tissue metal accumulation and biochemical analyses. In each experiment, D. magna were exposed for 48 h to the individual heavy metals, either alone or in combination

with the fungal pigment. An untreated control group and a fungal pigment-only group were included in each experiment. Each treatment consisted of three independent replicates containing ten individuals per replicate.

Analytical Procedures and QA/QC

Heavy metal concentrations in the test solutions and tissue samples were quantified using an Agilent 7800 ICP-MS (Agilent Technologies, Tokyo, Japan) at the Eastern Anatolia High Technology Application and Research Center (DAYTAM), Atatürk University, Erzurum, Türkiye. The instrument was operated under standard plasma conditions and calibrated using multi-element calibration standards (High-Purity Standards, USA). To ensure the accuracy and precision of the analytical results, certified reference materials (CRMs) were analyzed alongside the samples. The limits of detection (LOD) and quantitation (LOQ) were determined based on the standard deviation of ten reagent blanks. For the analyzed metals (Cd, Zn, Cr, Hg, and Cu), the LOD values were consistently below 0.01 µg/L, whereas the LOQ values were below 0.05 µg/L. All analytical procedures followed strict quality assurance/quality control (QA/QC) protocols, including the use of high-purity reagents and periodic calibration verification throughout the analytical process.

Biochemical Analysis

At the end of each independent exposure experiment, D. magna specimens were pooled within each replicate to obtain sufficient tissue mass. Replicates were not pooled with one another. The pooled specimens were blotted dry, weighed, and homogenized on ice in phosphate-buffered saline (PBS) at a ratio of 1:5 (w/v). The homogenates were centrifuged at 12,000 rpm for 15 min at 4 °C, and the resulting supernatants were collected and stored at −80 °C until biochemical analyses were conducted. The activities of superoxide dismutase (SOD) and catalase (CAT), as well as the levels of reduced glutathione (GSH) and malondialdehyde (MDA), were determined following the methodology described by Luo et al. (2025). SOD activity was quantified at 325 nm using the hydroxylamine method as described by Oyanagui (1984). CAT activity was measured spectrophotometrically at 240 nm according to the method of Zang et al. (2012). Lipid peroxidation was assessed by measuring MDA concentrations based on the thiobarbituric acid (TBA) reaction at 532 nm, following the protocol of Ohkawa et al. (1979). Cytosolic GSH levels were determined using a digitonin-permeabilization technique that selectively releases cytosolic components, as described by Gao et al. (2018). Metal accumulation in tissues was quantified using ICP-MS.

Statistical Analysis

All experiments were performed in triplicate, and results are presented as mean ± standard deviation (SD). Statistical analyses were conducted using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). The 48-h LC_{50} values were estimated by probit analysis. Because each heavy metal exposure experiment was conducted independently, statistical analyses were performed separately for each metal using one-way analysis of variance (ANOVA), followed by Tukey's HSD test for post hoc pairwise comparisons where appropriate. Within each metal-specific experiment, comparisons were made among the corresponding treatment groups, including the control, fungal pigment-only, metal-only, and metal + fungal pigment groups. Differences were considered statistically significant at $p < 0.05$.

Results

Characterization and Antioxidant Activity of the Fungal Pigment

The antioxidant activity of the fungal pigment extract derived from Fusarium tricinctum was evaluated using the DPPH free radical scavenging assay and compared with the standard antioxidant, ascorbic acid (Fig. 1). The pigment extract exhibited a concentration-dependent antioxidant activity, with DPPH radical scavenging increasing as the pigment concentration increased. At 100 µg/mL, the fungal pigment exhibited nearly complete (approximately 100%) DPPH radical scavenging activity, whereas approximately 50% inhibition was observed at 50 µg/mL.

The carotenoid composition of the pigment extract was characterized by HPLC and UV–Vis spectrophotometric analyses (Fig. 2). The HPLC chromatogram recorded at 450 nm revealed the presence of neurosporaxanthin, torulene, γ-carotene, ζ-carotene, β-zeacarotene, and β-carotene, which were identified based on their retention times and characteristic absorption profiles. Among these carotenoids, β-carotene exhibited the highest peak intensity, indicating that it was the predominant carotenoid in the extract. The UV–Vis absorption spectrum (350–600 nm) displayed three characteristic absorption maxima at approximately 420, 445, and 470 nm, which are consistent with the typical spectral characteristics of carotenoids containing extended conjugated double-bond systems. The agreement between the HPLC and UV–Vis analyses confirmed the carotenoid composition of the fungal pigment extract, with β-carotene representing the major pigment component.

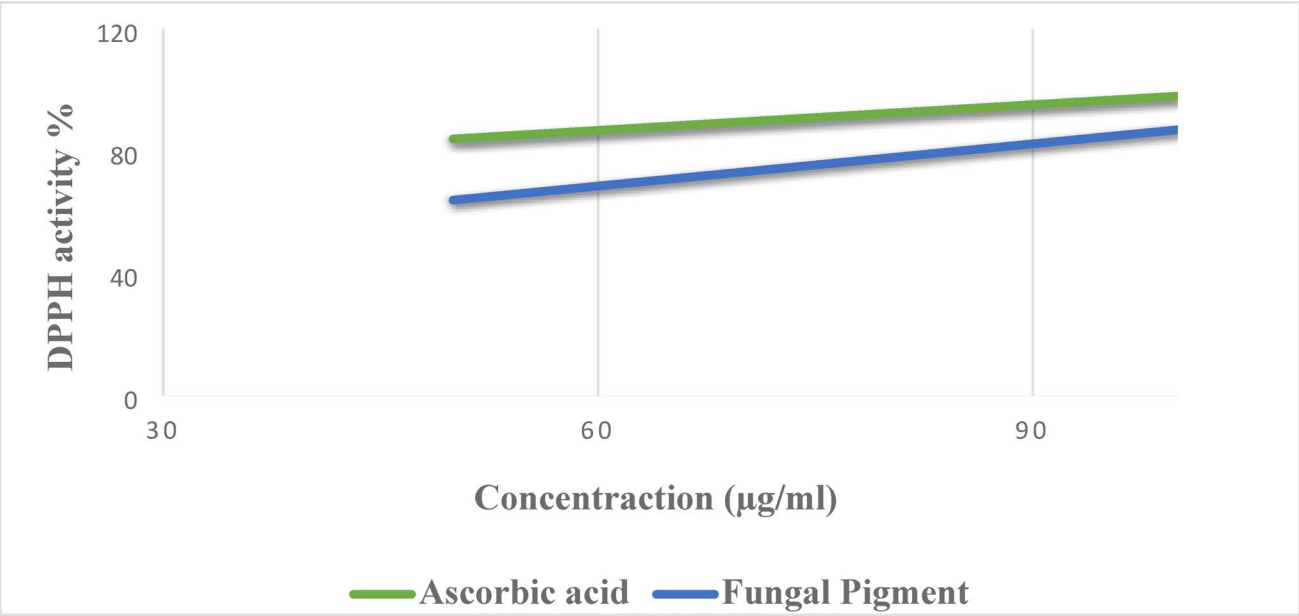

Fig. 1 Antioxidant activity of the fungal pigment extract derived from *F. tricinctum*

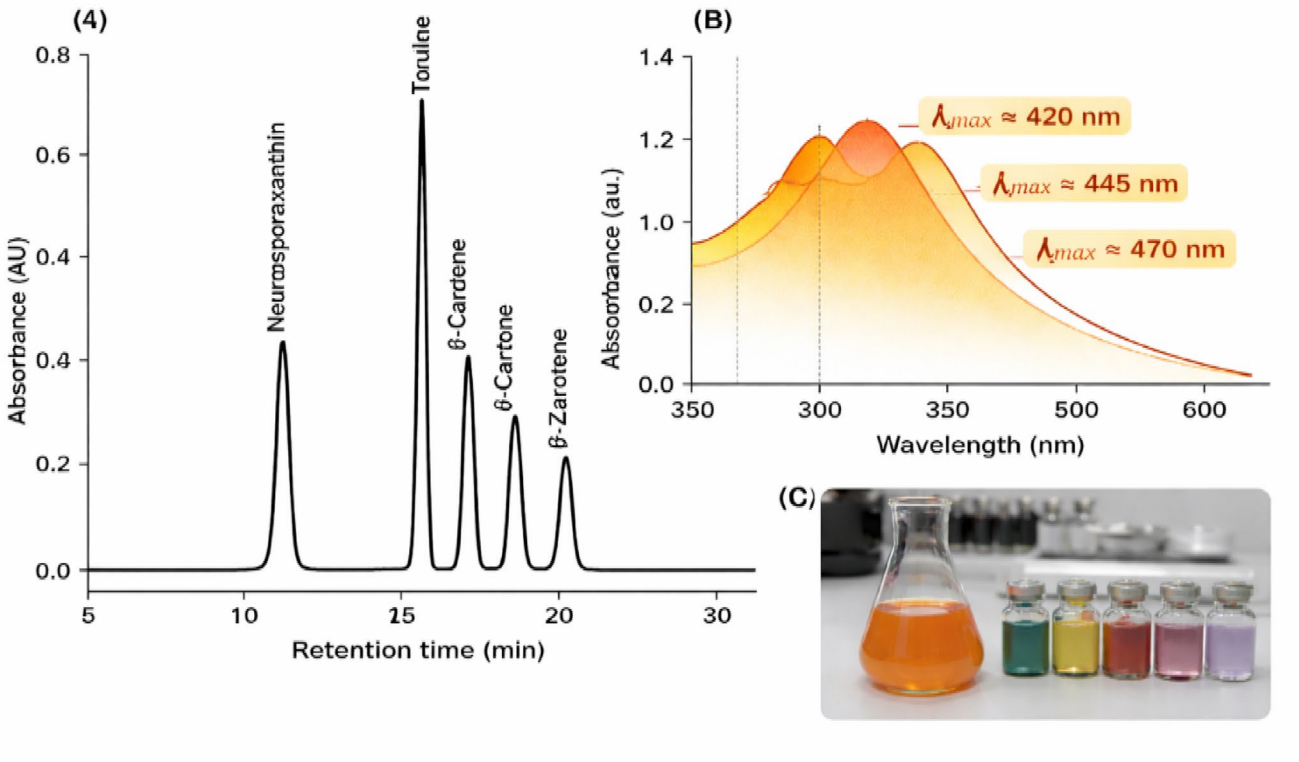

Fig. 2 Pigment profile of the extract obtained from *Fusarium tricinctum* as determined by HPLC and UV–Vis analyses

Heavy Metal Toxicity and LC_{50} Values

The effects of heavy metals at different concentrations (5–100 µg/L) on the survival of D. magna are presented in Fig. 3. A concentration-dependent increase in mortality was observed for all tested metals. Based on the 48-h LC_{50} values calculated by probit analysis, mercury (Hg) exhibited the highest toxicity, followed by chromium (Cr), copper (Cu), and cadmium (Cd), whereas zinc (Zn) showed the lowest toxicity.

The 48-h LC_{50} values differed substantially among the tested substances, with Hg showing the lowest LC_{50} and the fungal pigment showing the highest LC_{50} (Fig. 3B). Based on the immobilization responses observed across the tested concentration range, 10 µg/L was selected as the common sublethal concentration for subsequent experiments. At this concentration, immobilization remained limited while measurable responses were observed across the heavy metal treatments (Fig. 3A). The same 10 µg/L concentration was used for all heavy metals and the fungal pigment to enable direct comparison among treatments.

Co-exposure to the fungal pigment was associated with lower mortality than exposure to the corresponding heavy metal alone in all treatment groups. Mortality rates in the pigment-treated groups were 40% (Cd), 20% (Zn), 50% (Cr), 60% (Hg), and 30% (Cu) after 48 h of exposure. The pigment-only group exhibited low mortality throughout the experiment (10% at 24 h and 20% at 48 h) (Fig. 4).

Metal Accumulation

Although all heavy metals were applied at the same concentration (10 µg/L), ICP-MS analysis revealed significant differences in tissue metal accumulation among the treatment groups ($p < 0.05$). Low concentrations of the analyzed metals were detected in both the control and fungal pigment-only groups. Among the heavy metal-treated groups, tissue accumulation was highest in the Hg group, followed by Cu, Cr, Cd, and Zn. Co-exposure to the fungal pigment resulted in significantly lower tissue metal accumulation for all heavy metals compared with the corresponding metal-only groups ($p < 0.05$). No significant difference in tissue metal accumulation was observed between the control and fungal pigment-only groups (Table 1).

Biochemical Analyses

Malondialdehyde (MDA) levels are presented in Fig. 5. In the control group, MDA levels remained relatively stable, increasing slightly from approximately 7.0 nmol mg^{-1} at

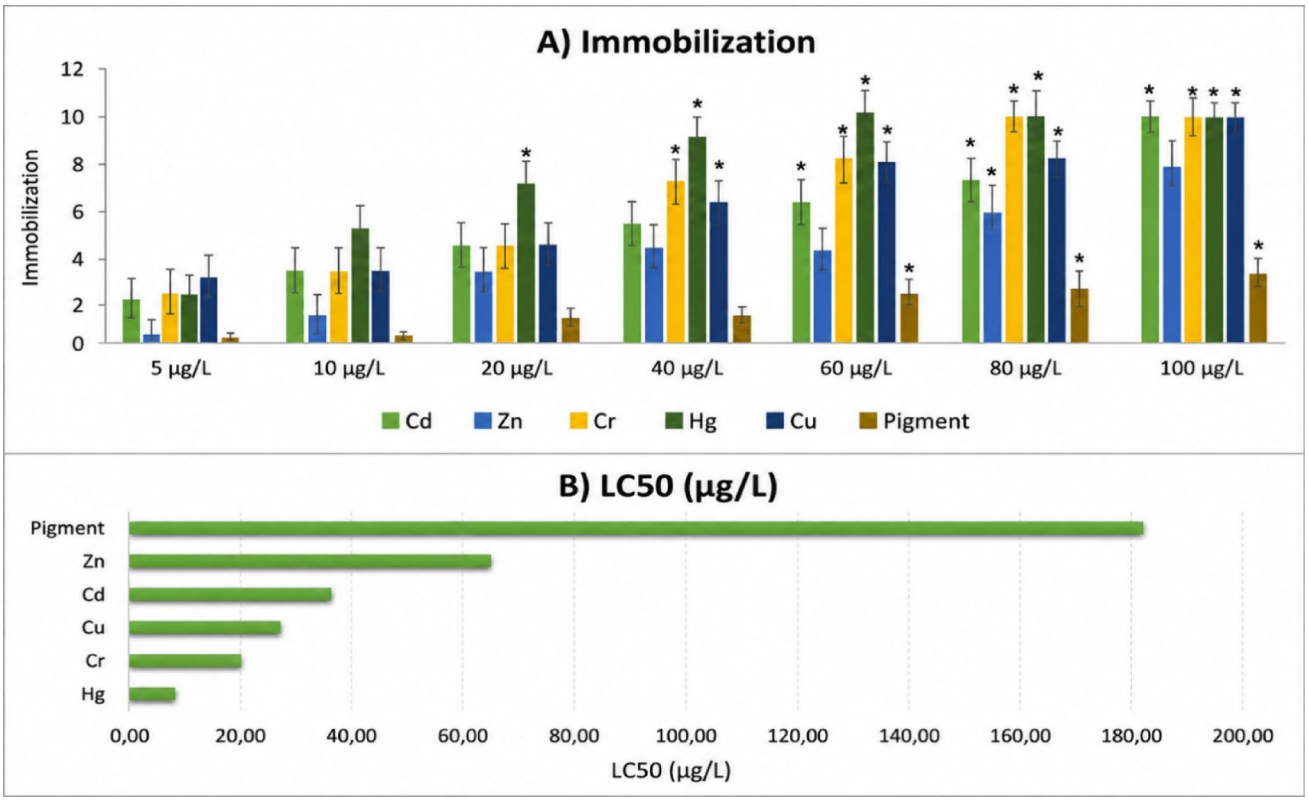

Fig. 3 Immobilization of Daphnia magna exposed to different concentrations (5–100 µg/L) of heavy metals and corresponding 48-h LC_{50} values. Data are presented as mean ± standard deviation (SD) (n = 3), and error bars represent standard deviation. Different symbols above the bars indicate statistically significant differences among groups within each metal exposure ($p < 0.05$)

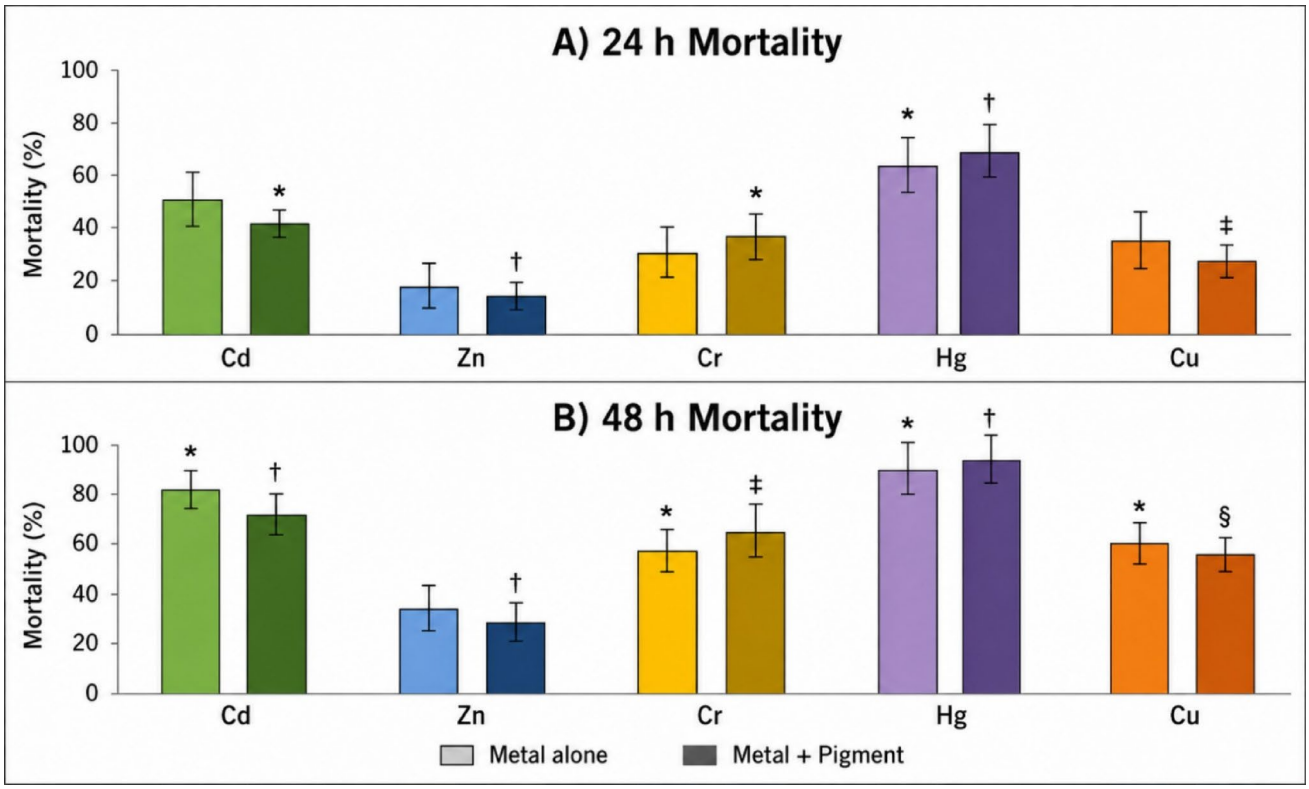

Fig. 4 Mortality rates of Daphnia magna exposed to individual heavy metals (Cd, Zn, Cr, Hg, and Cu) alone or in combination with the fungal pigment for 24 and 48 h. Data are presented as mean ± standard deviation (SD) (n = 3), and error bars represent standard deviation. Different symbols above the bars indicate statistically significant differences among treatment groups ($p < 0.05$)

24 h to 7.5 nmol mg^{-1} at 48 h. Exposure to all heavy metals significantly increased MDA levels compared with the control group, with the highest values observed in the Hg-treated group, followed by the Cr, Cu, Cd, and Zn groups. MDA levels were consistently higher after 48 h than after 24 h of exposure. Co-exposure to the fungal pigment was associated with lower MDA levels in all heavy metal-treated groups compared with the corresponding metal-only treatments. The fungal pigment-only group exhibited MDA levels close to those of the control throughout the experimental period (Fig. 5).

Following 48 h of exposure, all heavy metal treatments altered antioxidant enzyme activities relative to the control group (Table 2). SOD, CAT, and GSH activities generally increased following heavy metal exposure, with the strongest responses observed in the Hg-treated group. Elevated enzyme activities were also observed in the Cu, Cr, Cd, and Zn groups, although the magnitude of the response differed among the metals. Co-exposure to the fungal pigment was associated with lower SOD, CAT, and GSH activities than those observed in the corresponding heavy metal-only groups. The fungal pigment-only group exhibited enzyme activities comparable to those of the control group, indicating that pigment exposure alone did not substantially alter basal antioxidant enzyme activities.

Discussion

Heavy metal contamination remains one of the major environmental threats to aquatic ecosystems because these pollutants persist in the environment, accumulate in aquatic organisms, and promote excessive production of reactive oxygen species (ROS), ultimately leading to oxidative damage, lipid peroxidation, and disruption of normal cellular functions (Chupani et al. 2022; El-Deeb Ghazy et al. 2024; Cojocaru et al. 2025). In the present study, exposure of Daphnia magna to Cd, Zn, Cr, Hg, and Cu resulted in increased mortality, tissue metal accumulation, elevated lipid peroxidation, and alterations in antioxidant defense parameters. In contrast, co-exposure to the carotenoid-rich fungal pigment produced by Fusarium tricinctum was associated with lower mortality, reduced tissue metal accumulation, and lower oxidative stress biomarkers. Taken together, these findings indicate an association between fungal pigment exposure and reduced physiological stress under acute heavy metal exposure. However, the precise mechanisms underlying these responses were not directly investigated in the present study.

The fungal pigment exhibited remarkably high antioxidant activity, reaching nearly complete DPPH radical

Table 1 Tissue concentrations of metals in *D. magna* following exposure to individual heavy metals, with or without fungal pigment treatment, as determined by ICP–MS

Groups	Cd(ppm)	Zn(ppm)	Cr(ppm)	Hg(ppm)	Cu(ppm)
Control	0.10 ± 0.02^{e}	1.00 ± 0.12^{e}	0.15 ± 0.03^{e}	0.04 ± 0.01^{e}	0.80 ± 0.10^{e}
Fungal pigment	0.12 ± 0.03^{e}	1.05 ± 0.14^{e}	0.16 ± 0.04^{e}	0.05 ± 0.01^{e}	0.85 ± 0.11^{e}
Cd (10 µg/L)	2.40 ± 0.25^{a}	1.10 ± 0.15^{e}	0.18 ± 0.04^{e}	0.05 ± 0.01^{e}	0.90 ± 0.12^{e}
Cd + Fungal pigment	1.50 ± 0.18^{b}	1.05 ± 0.14^{e}	0.17 ± 0.03^{e}	0.05 ± 0.01^{e}	0.88 ± 0.11^{e}
Zn (10 µg/L)	0.15 ± 0.04^{e}	6.80 ± 0.65^{a}	0.20 ± 0.05^{e}	0.06 ± 0.02^{e}	1.00 ± 0.15^{e}
Zn + Fungal pigment	0.14 ± 0.03^{e}	4.20 ± 0.50^{b}	0.19 ± 0.04^{e}	0.06 ± 0.02^{e}	0.98 ± 0.14^{e}
Cr (10 µg/L)	0.16 ± 0.04^{e}	1.20 ± 0.18^{e}	4.90 ± 0.45^{a}	0.08 ± 0.02^{e}	1.10 ± 0.17^{e}
Cr + Fungal pigment	0.15 ± 0.03^{e}	1.15 ± 0.17^{e}	3.10 ± 0.35^{b}	0.07 ± 0.02^{e}	1.05 ± 0.16^{e}
Hg (10 µg/L)	0.18 ± 0.05^{e}	1.30 ± 0.20^{e}	0.25 ± 0.06^{e}	5.80 ± 0.55^{a}	1.20 ± 0.18^{e}
Hg + Fungal pigment	0.16 ± 0.04^{e}	1.25 ± 0.19^{e}	0.23 ± 0.05^{e}	3.60 ± 0.40^{b}	1.15 ± 0.17^{e}
Cu (10 µg/L)	0.14 ± 0.03^{e}	1.40 ± 0.22^{e}	0.22 ± 0.05^{e}	0.10 ± 0.03^{e}	7.20 ± 0.70^{a}
Cu + Fungal pigment	0.13 ± 0.03^{e}	1.35 ± 0.20^{e}	0.21 ± 0.04^{e}	0.09 ± 0.02^{e}	4.50 ± 0.55^{b}

Values are expressed as mean ± standard deviation (SD) (n = 3). Within each column, means bearing different superscript letters are significantly different according to one-way analysis of variance (ANOVA) followed by Tukey's HSD post hoc test ($p < 0.05$).

Table 2 Effects of individual heavy metals, alone or in combination with fungal pigment, on superoxide dismutase (SOD) and catalase (CAT) activities and reduced glutathione (GSH) levels in *D. magna*

Groups	SOD (U/mg prot.)	CAT (U/mg prot.)	GSH (U/mg prot.)
Control	85.0 ± 4.2^{d}	90.0 ± 3.8^{d}	88.0 ± 4.0^{c}
Fungal pigment	90.0 ± 3.9^{cd}	98.0 ± 4.1^{cd}	95.0 ± 3.6^{bc}
Cd	110.0 ± 5.1^{b}	113.0 ± 4.7^{b}	88.0 ± 4.3^{c}
Cd + Fungal pigment	100.0 ± 4.6^{c}	106.0 ± 4.2^{c}	85.0 ± 3.9^{c}
Zn	95.0 ± 4.0^{c}	100.0 ± 4.5^{c}	85.0 ± 3.7^{c}
Zn + Fungal pigment	90.0 ± 3.8^{cd}	95.0 ± 4.0^{cd}	82.0 ± 3.5^{c}
Cr	112.0 ± 5.3^{b}	108.0 ± 4.6^{b}	92.0 ± 4.1^{bc}
Cr + Fungal pigment	100.0 ± 4.4^{c}	100.0 ± 4.3^{c}	90.0 ± 3.8^{c}
Hg	145.0 ± 6.2^{a}	122.0 ± 5.0^{a}	100.0 ± 4.6^{a}
Hg + Fungal pigment	138.0 ± 5.8^{a}	118.0 ± 4.9^{a}	98.0 ± 4.2^{ab}
Cu	122.0 ± 5.5^{b}	110.0 ± 4.8^{b}	100.0 ± 4.4^{a}
Cu + Fungal pigment	115.0 ± 5.0^{b}	100.0 ± 4.2^{c}	97.0 ± 4.1^{ab}

Values are expressed as mean ± standard deviation (SD) (n = 3). Within each column, means sharing at least one superscript letter are not significantly different, whereas means with no superscript letter in common differ significantly according to one-way ANOVA followed by Tukey's HSD post hoc test ($p < 0.05$)

scavenging activity at the highest tested concentration. Similar antioxidant capacities have previously been reported for carotenoid-rich fungal pigments and have largely been attributed to their ability to quench singlet oxygen and neutralize free radicals (Rapaport et al. 2021; Afroz Toma et al. 2023; Naz et al. 2023). HPLC and UV–Vis analyses further demonstrated that the pigment extract consisted predominantly of carotenoid compounds, including neurosporaxanthin, torulene, γ-carotene, ζ-carotene, β-zeacarotene, and β-carotene, with β-carotene representing the dominant component. The characteristic absorption maxima observed at approximately 420, 445, and 470 nm are consistent with previously reported fungal carotenoid spectra (Maoka 2020). These carotenoids are recognized for their

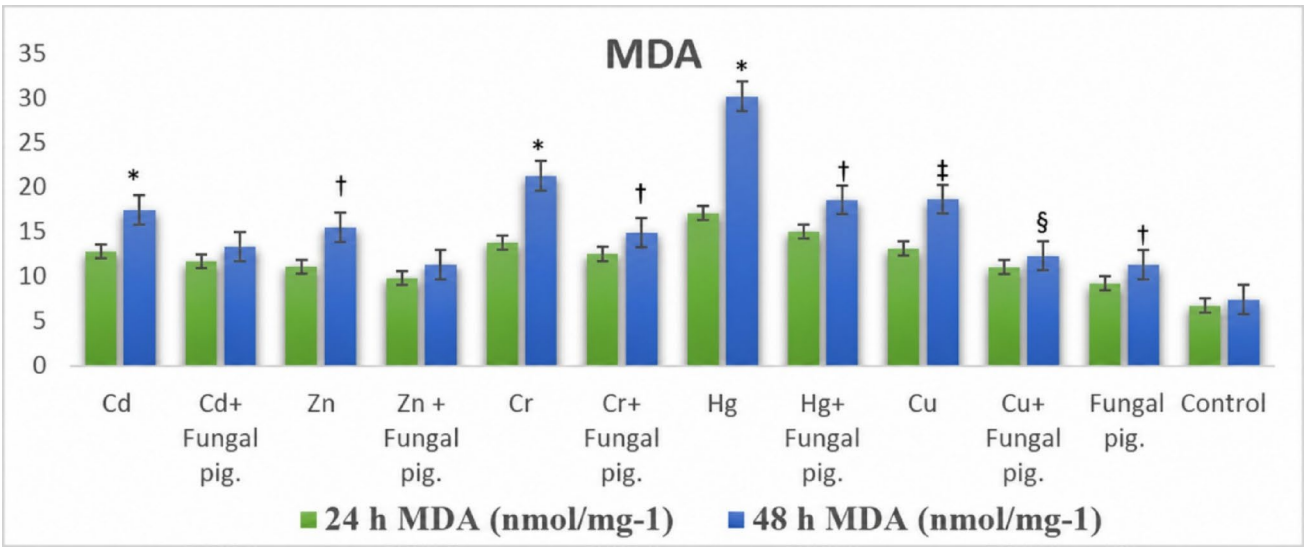

Fig. 5 Efffect of the fungal pigment on MDA levels in *D. magna* under heavy metal–induced oxidative stress. Data are presented as mean ± standard deviation (SD) (n = 3). Different symbols above the bars indicate statistically significant differences among treatment groups within each metal exposure ($p < 0.05$).

antioxidant capacity and their important roles in protecting biological membranes against oxidative injury by scavenging reactive oxygen species and maintaining cellular redox balance (Maoka 2020; Liu et al. 2023; Zhuang et al. 2022). Although complementary structural analyses such as LC–MS/MS or NMR would provide definitive structural confirmation, the chromatographic and spectroscopic evidence obtained in this study supports the carotenoid-rich nature of the fungal extract.

Among the tested metals, mercury exhibited the highest toxicity, whereas zinc produced the lowest toxic response. Similar toxicity patterns have been reported in previous ecotoxicological studies using Daphnia species and other freshwater invertebrates (Chupani et al. 2022; Paylar et al. 2024). Mercury possesses a particularly high affinity for sulfhydryl-containing proteins, interferes with numerous enzymatic processes, and promotes excessive ROS production, whereas zinc is an essential trace element whose toxicity generally becomes evident only at elevated concentrations (Queiroz and Torresi 2025; Cojocaru et al. 2025). These differences in metal-specific biological activity and toxicity provide a plausible explanation for the distinct mortality and oxidative stress profiles observed among the treatments.

The protective pattern observed following pigment co-exposure is particularly relevant when considered together with the antioxidant properties of the pigment. Carotenoids have long been recognized as efficient antioxidants capable of limiting oxidative damage under environmental stress conditions (Maoka 2020; Pereira da Costa and Miranda-Filho 2020). The lower mortality observed in pigment-treated groups is therefore consistent with the established antioxidant properties of carotenoid compounds. Nevertheless, survival alone cannot distinguish among possible protective processes. Because the present study did not directly examine molecular or physicochemical interactions between the pigment and heavy metals, it remains unclear whether the observed improvement in survival was primarily related to attenuation of oxidative stress, differences in tissue metal accumulation, or a combination of processes.

Lipid peroxidation, evaluated through MDA determination, increased markedly following heavy metal exposure, particularly in the mercury-treated group, indicating enhanced oxidative damage to cellular membranes. Elevated MDA concentrations are widely accepted as biomarkers of oxidative stress in aquatic organisms exposed to environmental contaminants (Liu et al. 2023; Luo et al. 2025). The lower MDA concentrations observed following pigment co-exposure suggest that the carotenoid-rich extract was associated with reduced lipid peroxidation under metal stress. This interpretation is consistent with previous reports describing the antioxidant properties of carotenoid-rich fungal pigments (Rapaport et al. 2021; Naz et al. 2023). Importantly, the present findings support an association between pigment exposure and reduced oxidative damage but do not establish a direct biochemical interaction between individual carotenoids and ROS.

Heavy metal exposure also altered antioxidant defense responses, as reflected by increased SOD, CAT, and GSH activities. Such responses are commonly interpreted as adaptive physiological mechanisms that protect organisms against excessive ROS production during metal stress (Zhuang et al. 2022; Liu et al. 2023). The stronger antioxidant responses observed in the mercury-treated group are consistent with its greater toxicity and oxidative challenge. In contrast, antioxidant enzyme activities in pigment-treated groups were generally closer to those measured in the control organisms. Rather than indicating direct modulation of antioxidant enzymes by the fungal pigment, this pattern may reflect a lower degree of oxidative challenge in pigment-treated organisms and, consequently, a reduced requirement for activation of endogenous antioxidant defenses. This interpretation remains tentative because direct measurements of ROS generation and antioxidant gene expression were not performed.

ICP-MS analyses demonstrated substantial differences in tissue metal accumulation among the investigated metals despite the use of the same nominal exposure concentration. Mercury accumulated to the greatest extent, whereas zinc exhibited the lowest accumulation, broadly corresponding to the observed differences in toxicity. Tissue metal concentrations were also lower in pigment-treated groups than in the corresponding metal-only treatments. These findings indicate that pigment exposure was associated with altered tissue metal accumulation; however, they do not establish whether the pigment directly affected metal uptake, transport, binding, intracellular distribution, or elimination. In particular, the present study did not include experiments specifically designed to distinguish between adsorption, altered uptake, or enhanced elimination. Therefore, the lower tissue concentrations should not be interpreted as direct evidence of metal chelation or reduced metal bioavailability. Additional experiments addressing metal transport pathways, adsorption behavior, and intracellular metal distribution will be required to clarify the mechanism underlying this observation.

An important consideration is that the same 10 µg/L exposure concentration was applied across the different metals and pigment to facilitate comparative assessment. This concentration should therefore be interpreted as a common exposure level rather than an equivalent toxicological threshold, because the tested metals exhibited markedly different acute toxicities. The particularly high toxicity of mercury emphasizes the importance of considering metal-specific

dose–response relationships in future studies. The present study was also limited to acute 48-h exposure conditions and focused on survival, tissue metal accumulation, and selected biochemical biomarkers. Consequently, the findings provide an initial assessment of the protective association of the fungal pigment under acute metal stress rather than evidence of long-term environmental efficacy. Future studies incorporating chronic exposure experiments, dose–response designs, transcriptomic or proteomic analyses, and more comprehensive pigment characterization would help clarify the molecular mechanisms involved and determine whether the observed responses persist under environmentally relevant exposure conditions.

Overall, the present findings indicate that carotenoid-rich pigment produced by Fusarium tricinctum was associated with improved survival, lower oxidative stress biomarkers, and reduced tissue metal accumulation in Daphnia magna exposed to heavy metals. The concordance among survival, MDA, antioxidant defense, and tissue metal responses suggests that the pigment may have protective potential under acute metal stress. However, the present evidence does not establish a specific mode of action, metal-chelation capacity, or reduction in metal bioavailability. Further mechanistic and chronic-exposure studies are therefore necessary to determine the biological basis and environmental relevance of these observations.

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Author Contributions Ozlem Gulmez Aksak and Hatice Dane contributed equally to the conception and design of the study, data acquisition, analysis and interpretation, and manuscript preparation. Both authors read and approved the final manuscript.

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Data Availability All data generated or analysed during this study are included in this published article and its supplementary information files.

Declarations

Conflict of Interest The authors declare that they have no known conflict of interest that could have appeared to influence the work reported in this paper.

Consent to Participate This is not applicable.

Consent to Publiscation This is not applicable.

Ethical Approval This study did not require approval from a research ethics committee because the experimental work was conducted using an unregulated invertebrate species (Daphnia magna).

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