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Comet Assay Evaluation of Cadmium Chloride-Induced DNA Damage in *Cyprinus carpio* (Common Carp) and the Genoprotective Role of Selenium

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ABSTRACT

Heavy metal contamination in aquatic environments represents a significant threat to biodiversity and the sustainability of aquatic resources, with cadmium being among the most toxic pollutants owing to its high bioaccumulation potential and genotoxic effects. This study investigated the genotoxicity induced by cadmium chloride (0.78 ppm and 1.56 ppm) in *Cyprinus carpio* (common carp). The ameliorative efficacy of selenomethionine (0.25 and 0.50 ppm) as a genoprotective agent was evaluated. The comet assay, a widely used technique in genetic toxicology, was employed to quantify DNA damage in fish tissues, providing sensitive and reliable measures of genotoxic effects. The results revealed a dose-dependent increase in DNA strand breaks following cadmium chloride exposure, indicating significant genotoxicity. Conversely, co-treatment with selenomethionine notably reduced DNA damage, highlighting its potential to mitigate Cd-induced genotoxicity. These findings enhance our understanding of heavy metal toxicity and instill hope for the potential use of selenomethionine as a sustainable intervention to protect aquatic life. From a societal perspective, protecting fish health is crucial for global food security and the well-being of millions who rely on fisheries for their livelihood. The protective capacity of selenium underscores the promise of sustainable, environmentally safe interventions to combat pollution, foster ecological resilience, and preserve natural resources for future generations. This study identifies knowledge gaps and provides a comprehensive understanding of DNA damage assessment through the prism of the comet assay, highlighting the protective role of selenium in alleviating Cd-induced genotoxicity.

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INTRODUCTION

Freshwater resources are essential for sustaining life and maintaining an ecological balance. However, rapid industrialization, urbanization, population growth, and unsustainable exploitation of natural resources have significantly degraded water quality (Carolin et al. 2017, Joseph et al. 2019, Qiao et al. 2020, Vardhan et al. 2019, Sharma et al. 2024, Saravanan et al. 2024). Heavy metals are particularly concerning among the major pollutants because of their persistence, toxicity, and bioaccumulative nature (Niu et al. 2020, Singh et al. 2011, Tchounwou 2012, Wu et al. 2016). Cadmium (Cd), widely used in industrial processes, is one of the most toxic heavy metals and frequently enters aquatic ecosystems through specific activities such as mining, smelting, electroplating, battery production, and the use of phosphate fertilizers. For instance, Cd can be released into the environment by extracting and processing ores during mining. Similarly, in battery production, cadmium is a common component of rechargeable batteries, and its disposal can lead to environmental contamination (Khan et al. 2022, Das et al. 2023, Rahoui et al. 2014, Irfan et al. 2021, Hayat et al. 2019, Kubier et al. 2019).

Owing to its high solubility and mobility, Cd easily accumulates in aquatic organisms, especially fish, posing severe and potentially catastrophic health risks to aquatic life and humans through the food chain (Mielcarek et al. 2022). It disrupts

vital cellular processes, such as DNA replication, repair, and apoptosis, causing oxidative stress and elevated reactive oxygen species (ROS) levels, resulting in DNA strand breaks and mutations (Liao et al. 2021, Liu et al. 2017, Qu & Zheng 2024, Hakem 2008, Wang 2015). Chronic exposure leads to cancer and systemic toxicity in multiple organs (Taysı 2024, Obaiah et al. 2020, Ferro et al. 2021, Noor et al. 2020, Zheng et al. 2021). Even at low concentrations, Cd exhibits genotoxicity and is classified as a Group 1 human carcinogen (IARC 1993), underlining the gravity of the situation.

The common carp (*Cyprinus carpio*), a widely consumed and economically important freshwater fish, is an ideal bioindicator because of its physiological adaptability (Mancera-Rodríguez et al. 2024). Selenium (Se), a trace element with antioxidant properties, plays a critical role in cellular defense against oxidative damage (Kora 2018, Li et al. 2023). In its organic form, selenomethionine (SeMet), Se enhances antioxidant enzyme activity and mitigates oxidative stress-induced DNA damage (Wenfi Jia 2023, Marieke Swinkels 2020).

This study advances the current understanding of aquatic toxicology by providing detailed evidence of Cd-induced genotoxicity in *C. carpio* and evaluating the protective efficacy of SeMet across multiple tissues using the comet assay. While earlier studies have established the bioaccumulation and genotoxic potential of Cd in fish (Cuypers et al. 2010, Pandey et al. 2011), limited research has comprehensively assessed tissue-specific DNA damage patterns alongside the mitigating effects of selenium-based antioxidants. By demonstrating that SeMet significantly reduces genotoxicity in a dose-dependent manner, particularly in gills, kidneys, and blood, this study builds on prior work (Elia et al. 2011, Kieliszek & Błażejak 2013) and addresses knowledge gaps regarding the role of selenium in oxidative stress regulation and DNA repair. Furthermore, the validation of the Genetic Damage Index (GDI) as a sensitive metric enhances the methodological framework for genotoxicity screening. These findings may contribute significantly to the existing literature advocating the integration of antioxidant-based interventions in pollution mitigation and aquaculture health management.

MATERIALS AND METHODS

Experimental Setup and Chemicals

Juvenile *C. carpio* (single breed) with an average length of 12.52 ± 2.00 cm and a mean weight of 24.00 ± 2.00 g were obtained from the Tamil Nadu Fisheries Development Corporation Limited (TNFDC), Aliyar, located in Coimbatore district, a renowned source of high-quality fish for research. The fish were transported to the laboratory in oxygenated water tanks to ensure safe handling of the fish. Upon arrival, they underwent prophylactic treatment by immersion in a 0.05% potassium permanganate ($KMnO_4$) solution for 2 min, repeated twice, to prevent dermal infections. Fish were acclimated for one month under controlled laboratory conditions following the protocol described by Palaniappan & Karthikeyan (2022). Waste materials, including fecal matter, were siphoned out daily to minimize the ammonia content in the tanks (Company et al. 2010). The fish were fed once daily with boiled eggs and minced goat liver ad libitum at 3% of their body weight. This diet was uniformly provided to both the control and exposed groups and was rapidly consumed, minimizing the possibility of food soaking in contaminated water.

Water quality parameters were meticulously monitored and consistently maintained within optimal ranges throughout the experimental period: temperature at 26.7 ± 1.6 °C, dissolved oxygen between 6.5–8.5 $mg.L^{-1}$, pH from 6.5 to 7.5, nitrite concentrations of 0.06–0.1 $mg.L^{-1}$, nitrate between 1–3.5 $mg.L^{-1}$, total hardness at 154 ± 1.7 $mg.L^{-1}$ (as $CaCO_3$), and total ammonia levels between 0.1–0.3 $mg.L^{-1}$. All exposures were conducted under natural photoperiod conditions. All chemicals used were of analytical grade and obtained from Himedia, India

Experimental Design for Lethal Toxicity Tests

After a month of acclimatization, healthy adult common carp weighing 45-50 grams were randomly assigned to acclimatization groups, with 10 fish per group. The study comprised nine experimental groups randomly assigned to treatment groups, with 10 fish per group, including a control group with no exposure (1 fish). The other groups are a cadmium chloride ($CdCl_2$) group exposed to sub-lethal concentrations of 0.78 ppm (2) and 1.56 ppm (3), a SeMet-only group exposed to either 0.25 ppm (4) and 0.50 ppm SeMet (5), co-exposure groups treated with 0.78 ppm $CdCl_2$ plus 0.25 SeMet (6) and 0.50 ppm SeMet (7), and another set of co-exposure groups treated with 1.56 ppm $CdCl_2$ plus 0.25 SeMet (7) and 0.50 ppm SeMet (8) based on prior studies (Ta et al. 2018, Mechlaoui et al. 2019), in a semi-static system with the change of test water every day to maintain the concentration of the chemical. The selection of concentrations 0.25 and 0.50 ppm for SeMet was based on the earlier work done by Elia et al. (2011). This resulted in nine distinct experimental conditions. Considering the five time intervals, each treatment condition involved 50 fish (9 × 10 fish × 5 time intervals). In the present study, water was used as a vehicle control, as it is a neutral substance that does not contain any selenium. All treatments were conducted in

triplicate and analyzed at five time intervals: 24, 48, 72, 96, and 120 h. At each time point, the animals were euthanized, and samples from the gills, liver, kidney, and peripheral blood were collected.

Cell Isolation and Preparation

Cell isolation and preparation were performed with utmost care and precision. *C. carpio* specimens wereanesthetized using clove oil (AQUI-S, Aquatic Anaesthetic, Aqua World LTD, India) at a concentration of 0.05 $mL.L^{-1}$ for 2–3 minutes, following the protocol described by Husen & Sharma (2015). Blood samples were collected aseptically from the caudal vein using heparinized syringes and immediately transferred to labeled microtubes. After anesthesia, the gill arches, liver, and kidneys were carefully excised using sterile scalpels. The excised tissues were thoroughly rinsed three times with sterile phosphate-buffered saline (PBS) to eliminate blood, debris, and surface contaminants. Clean tissues were placed into labeled Petri dishes and finely minced with sterile scalpels to create a homogeneous tissue suspension, from which any solid fragments were carefully removed using a pipette.

The homogenized tissue suspensions were transferred into labeled microtubes using sterile tips and incubated with 10 mL of 0.25% trypsin-EDTA solution (Merck, Merck Specialities Pvt. Ltd., Mumbai, India) to facilitate enzymatic dissociation. The tubes were placed on a rotating platform at ambient temperature for 10 min. Enzymatic activity was halted by adding 5 mL of fetal calf serum (FCS) to each microtube. Subsequently, the cell suspensions were transferred to fresh, labeled microtubes and centrifuged at 800 rpm for 10 min in a pre-cooled bench-top centrifuge. After centrifugation, the supernatant was discarded, and the resulting cell pellets were resuspended in 10 mL of FCS. All procedures were performed on ice to preserve cell viability and prevent thermal degradation, as recommended by Klobučar et al. (2012).

Comet Assay

Subsequently, the coverslip was carefully removed, and the slides were immersed in a freshly prepared lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100, pH 10) for 1 h to facilitate cellular lysis. The slides were then gently rinsed with redistilled water and placed in a horizontal electrophoresis chamber containing cold electrophoresis buffer (1 mM EDTA and 300 mM NaOH, pH 13). Slides were incubated in the buffer for 40 min at 4°C before electrophoresis, which was carried out at 1 $V.cm^{-1}$ and 300 mA for 25 min to allow DNA unwinding. After electrophoresis, the slides were rinsed thrice with a neutralization buffer containing 0.4 M Tris (pH 7.5). All procedures were performed under dim yellow light to prevent light-induced damage to the DNA. Slides were stained with 80 µL of ethidium bromide (20 $\mu g.mL^{-1}$) for 5 min, rinsed with cold distilled water to remove excess stain, air-dried, and mounted with coverslips. DNA migration patterns were visualized using a fluorescence microscope (NIKON Eclipse 400). DNA damage was evaluated using a subjective visual scoring system (scoring 100 nuclei per fish). Undamaged cells appeared with intact nuclei, whereas DNA-damaged cells exhibited a characteristic 'comet' appearance, with the tail representing fragmented DNA. Cells exhibiting no heads or fully dispersed heads, which are indicative of apoptosis, were excluded from the analysis.

The degree of DNA damage was determined with great care by classifying cells into five categories based on the extent of tail migration: Type 0 (no damage), Type I (low damage), Type II (moderate damage), Type III (serious damage), and Type IV (extensive damage). This visual scoring method allowed for semi-quantitative genotoxicity assessment (Grover et al. 2003).

Owing to the requirement of a large number of fish across multiple time points and replicates, additional positive and negative control groups were not included in this study to maintain ethical and logistical feasibility.

Statistical Analysis

DNA damage was assessed using the alkaline comet assay, scoring 100 cells per fish, and classifying them into five categories based on tail length (types 0–IV). The percentage of DNA-damaged cells (Types II–IV) and the genetic damage index (GDI) were calculated for each tissue (Collins et al. 2023). A thorough statistical analysis was conducted using one-way ANOVA followed by Tukey's HSD post-hoc test, with significance set at $p < 0.05$, to ensure the robustness of our conclusions.

$$GDI = (\text{Type 1} + \text{Type II} + \text{Type III} + \text{Type IV})/(\text{Type 0} + \text{Type I} + \text{Type II} + \text{Type III} + \text{Type IV}) \quad \ldots(1)$$

$$\%\ \text{of DNA damage} = \text{Type II} + \text{Type III} + \text{Type IV} \quad \ldots(2)$$

RESULTS AND DISCUSSION

This study explored the genotoxic effects of $CdCl_2$ and the protective role of SeMet in *C. carpio*. An alkaline comet assay was employed to evaluate DNA damage in the gill, liver, kidney, and blood tissues. The results revealed a significant, dose- and time-dependent increase in DNA damage in all tissues following Cd exposure, whereas SeMet demonstrated an apparent protective antioxidant effect.

In molecular biology and environmental science, DNA isolation of DNA constitutes a fundamental aspect of genetic research. Genotoxicity assays are critical for evaluating the effects of environmental stressors on aquatic organisms. Among the wide variety of aquatic life, fish are particularly significant as bioindicators, providing valuable insights into the ecological health of their habitats and the consequences of pollution. This underscores the importance of acquiring high-quality DNA from fish tissues and assessing DNA damage using methodologies such as the comet assay, which is vital for the conservation of aquatic resources.

Fish, widely acknowledged as effective bioindicators for evaluating metal pollution in aquatic ecosystems, played a pivotal role in this study. *C. carpio*, chosen as the model species, is prominent in aquaculture, frequently used in toxicological research, and serves as a sentinel organism due to its broad geographical distribution and sensitivity to environmental stressors (Forouhar Vajargah et al. 2018, Farhangi & Jafaryan 2019, García-Medina et al. 2022, Yancheva et al. 2022).

The alkaline comet assay, a crucial tool in this study, was initially developed by Singh et al. (1988) and adapted for use in fish erythrocytes. This is a sensitive method for detecting DNA strand breaks (Jiang et al. 2023). This assay has been extensively used across various tissues, including the gills, liver, and blood, for both *in vivo* and *in vitro* assessments following exposure to xenobiotics (Bajpayee et al. 2016). Blood is commonly used for genotoxicity testing because of its accessibility and the predominance of red blood cells (RBCS). Solid tissues, such as the liver and kidney, require careful dissociation to preserve DNA integrity and avoid artifact formation (Collins et al. 2023, Jha 2023).

The gill, liver, kidney, and blood tissues of the control group showed the maximum number of Type 0 and Type I nucleoids (Fig. 5A). The tissues of the exposed group showed the presence of Type II, Type III, and Type IV nucleoids, along with Type 0 and Type I nucleoids (Fig. 5B).

DNA Damage in Gill Nucleoids

The results of this study demonstrate that $CdCl_2$ exerts statistically significant genotoxic effects on the gill tissue of *C. carpio*, as reflected by the elevated percentage of DNA-damaged cells and GDI. The observed increase in DNA damage was both dose- and time-dependent, with the highest levels recorded at 1.56 ppm after 120 h of exposure ($GDI = 0.387$, $p < 0.05$, Fig. 1). These findings are consistent with earlier research showing that Cd exposure compromises DNA integrity in fish gills, primarily due to oxidative stress-induced strand breaks and impaired DNA repair mechanisms (Pandey et al. 2011, Ghosh & Indra 2018). Javed et al. (2016) found that thermal power plant effluent leads to concomitant damage to DNA in the gill and liver of the fish *C. punctatus*. A significantly higher mean tail length was observed in the exposed group compared to that in the control group.

As the primary site of metal uptake in aquatic organisms, the gills are particularly vulnerable to waterborne toxicants such as Cd. Their large surface area, rich vascularization, and direct contact with the external environment facilitate rapid accumulation of metals. Cd disrupts cellular homeostasis, interferes with calcium signaling, and induces ROS, leading to oxidative DNA damage (Genchi et al. 2020). The significant increase in GDI values in the Cd-exposed groups confirmed the extent of nuclear damage and supported its utility as a sensitive quantitative index for genotoxicity assessment. GDI values, which represent the percentage of DNA-damaged cells, are crucial for understanding the genotoxic effects of Cd. Importantly, the non-genotoxic nature of SeMet was validated by the low GDI values in SeMet-alone treatments, which remained statistically indistinguishable from the controls ($p > 0.05$).

The co-exposure groups demonstrated the protective role of SeMet against Cd-induced genotoxicity. Notably, 0.50 ppm SeMet significantly reduced ($p < 0.05$) DNA damage in gill tissues across all time points, with the most marked reduction observed at 96 h, where the GDI dropped from 0.385 in (Cd-alone group) to 0.146 (the SeMet co-treated group). This effect is attributed to the ability of SeMet to enhance antioxidant defenses by activating glutathione peroxidase and other selenoproteins that neutralize ROS and prevent oxidative DNA damage (McKelvey et al. 2015, Tchounwou et al. 2012). The greater reduction at higher SeMet doses suggests a dose-dependent protective effect. The antioxidant properties of SeMet, such as suppression of lipid peroxidation and stabilization of cellular membranes, further contribute to its genoprotective function (Hashtjin et al. 2024).

The dose-dependent efficacy of SeMet in mitigating DNA damage highlights its therapeutic potential. The 0.50 ppm dose consistently outperformed the 0.25 ppm dose, leading to a marked reduction in GDI values (Fig. 1). This emphasizes the role of SeMet as a powerful antioxidant and cytoprotective agent. These findings support the hormetic nature of selenium, which offers protective effects at low doses but may become toxic at higher concentrations (Angelone et al. 2024). Importantly, the significant reduction ($p < 0.05$) in genotoxicity with SeMet co-treatment instills confidence in its applicability in pollution mitigation strategies, reinforcing its potential for sustainable aquaculture health management.

While SeMet co-treatment showed a reduction in genotoxic damage compared to the Cd-only groups, in many

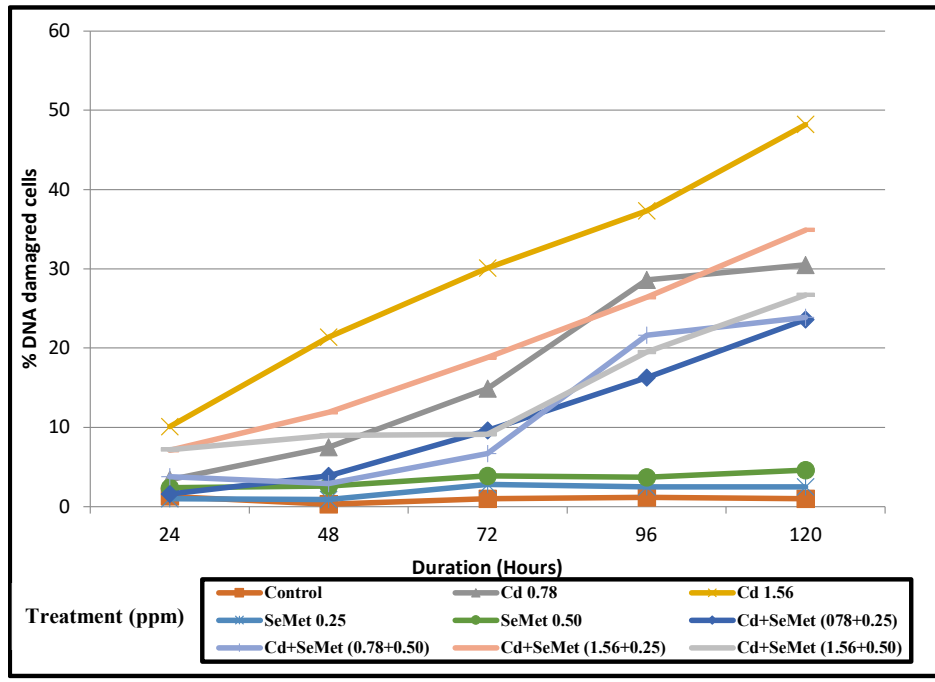

Fig. 1: Percentage of DNA damage in the gills of *C. carpio*.

instances, the damage levels remained significantly elevated relative to the controls. For example, in liver tissues at 120 h, the GDI values remained high despite SeMet exposure, indicating only partial rather than substantial protection. Thus, the genoprotective effect of SeMet could be interpreted as a moderate attenuation of genomic integrity.

DNA Damage in Liver Cells

The liver, a central organ for detoxification and metabolic processing, is highly vulnerable to pronounced DNA damage following $CdCl_2$ exposure. The percentage of DNA-damaged cells and the GDI increased in a precise dose- and time-dependent manner, with the most severe genotoxic effects recorded in the 1.56 ppm group at 120 h. This vulnerability, underscored by the liver's pivotal role in metal accumulation and oxidative stress regulation (Minarik et al. 2014, Ardeshir et al. 2017), highlights the urgent need for further research in this area. Cd exerts genotoxic effects primarily by generating ROS, depleting intracellular antioxidants such as glutathione, and disrupting DNA repair pathways (Cuypers et al. 2010).

The co-administration of SeMet offers a promising solution to address Cd-induced hepatic DNA damage. At 72 h, the higher SeMet dose (0.50 ppm) significantly reduced DNA damage from 27.0% to 9.8% ($p < 0.05$), demonstrating its potential as an effective antioxidant *via* activation of selenoenzymes such as glutathione peroxidase (Kieliszek & Błażejak 2013). However, this protective effect is not permanent, and elevated DNA damage persists. The unexpectedly high GDI value (0.590) (Fig. 2) observed in the Cd+SeMet (1.56+0.25 ppm) group at 120 h suggests that lower SeMet doses may be insufficient to counteract Cd toxicity or may alter metal retention and distribution within hepatic tissue (Wang et al. 2024). As a selenium-containing amino acid, SeMet serves as a precursor for key selenoproteins, such as GPx and thioredoxin reductase, which are essential for neutralizing ROS and maintaining cellular redox homeostasis (Zuo et al. 2019, Wande et al. 2020). These findings reveal the sensitivity of the liver to heavy metal damage, emphasizing the importance of ongoing research on better antioxidant treatments. While SeMet shows great potential in protecting our genes, its success depends on determining the correct dose and timing. This compelling dose-dependent response illustrates the hormetic nature of selenium, demonstrating its protective benefits at low concentrations while emphasizing the necessity of optimal levels to ensure safety and efficacy.

DNA Damage in Kidney Nucleoids

The kidney, an essential organ responsible for osmoregulation and excretion, exhibited significant genotoxic responses following CdCl2 exposure. DNA damage, assessed by the percentage of damaged cells and GDI, increased in a precise dose- and time-dependent manner. The most severe DNA damage was observed in the 1.56 ppm Cd group at 120 h, where 48.2% of the kidney cells exhibited DNA damage. The

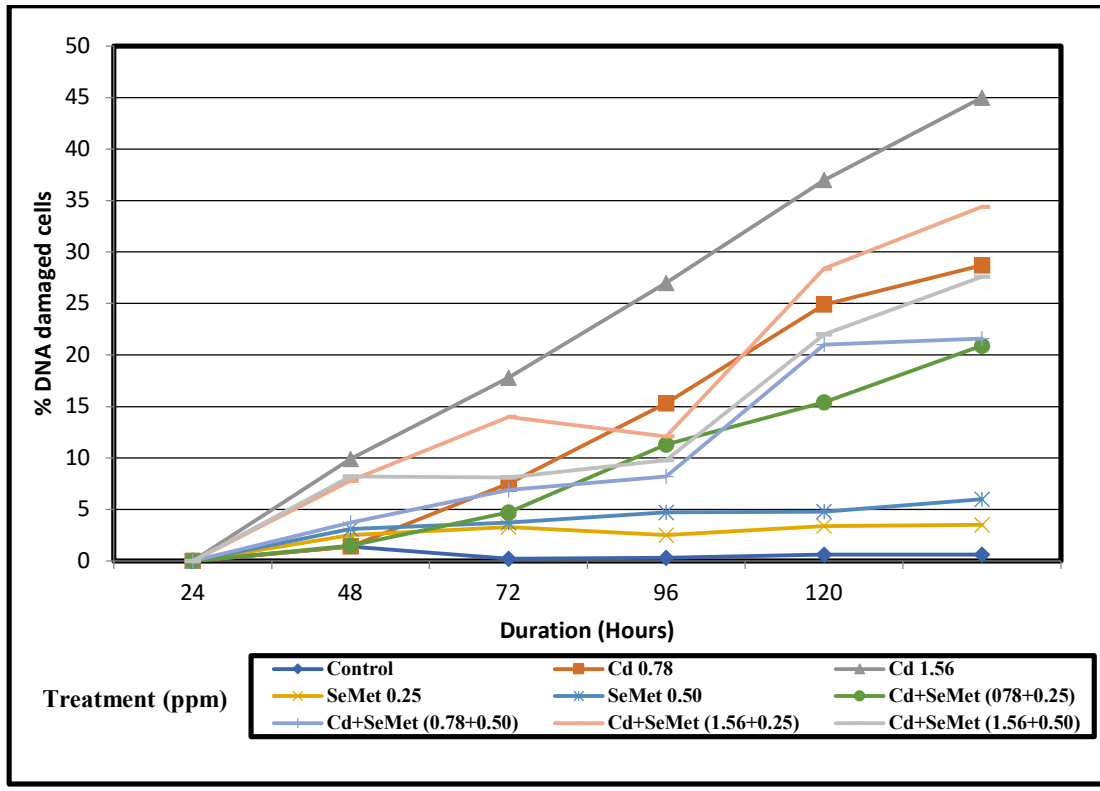

Fig. 2: Percentage of DNA damage in the liver of *C. carpio*.

GDI peaked at 0.568 (Fig. 3). These values were significantly elevated compared to those of the control group, which showed only 1.0% damage and a GDI of 0.044 ($p < 0.05$).

At earlier exposure durations, $CdCl_2$ elicited pronounced genotoxic effects. Specifically, at 72 h, fish exposed to 1.56 ppm Cd exhibited 30.1% DNA damage and a GDI of 0.372, while those treated with 0.78 ppm showed 14.9% damage and a GDI of 0.286 (Fig. 3). Both values were significantly elevated compared to those in the control group ($p < 0.05$), underscoring the potent genotoxicity of Cd. These findings are consistent with earlier studies indicating that Cd induces DNA strand breaks and oxidative base lesions, primarily through ROS and disruption of DNA repair mechanisms (Cuypers et al. 2010, Thévenod 2009). The pronounced accumulation of Cd in renal tissues is attributed to its high affinity for metallothioneins, which prolongs its retention and enhances its nephrotoxic impact.

Selenomethionine significantly ameliorated Cd-induced DNA damage in most treatment groups. At 72 h, co-exposure to Cd at 0.78 ppm and SeMet at 0.25 ppm resulted in a notable reduction in DNA damage from 14.9% to 9.6%, along with a decline in the GDI from 0.286 to 0.180 ($p < 0.05$). Similarly, at 120 h, the combination of Cd at 1.56 ppm with SeMet at 0.50 ppm led to a marked decrease in DNA damage from 48.2% to 26.7%, with a corresponding reduction in the GDI from 0.568 to 0.313, underscoring an apparent dose-dependent protective effect ($p < 0.05$). Nonetheless, SeMet did not afford complete genoprotective efficacy in all scenarios of this study. At 96 h, the group receiving 1.56 ppm Cd with 0.25 ppm SeMet still manifested appreciable DNA damage (26.4%) and a GDI of 0.299. Although these values were significantly lower than those in the Cd-only group (37.3% and 0.430, respectively), they remained markedly elevated relative to the control values ($p < 0.05$). These observations emphasize that SeMet imparts notable antioxidant and cytoprotective benefits, particularly at higher concentrations, and abrogates Cd-induced genotoxic effects, especially under suboptimal dosing or prolonged exposure.

The findings of this study are of utmost importance, as they underscore the superior sensitivity of the GDI as an indicator of DNA fragmentation severity compared with the percentage of damaged cells alone. While the percentage of damage provides insight into the incidence of genotoxic events, GDI offers a more refined assessment by quantifying the degree and extent of DNA migration, thereby providing a deeper understanding of the magnitude of damage (Kumar et al. 2010). The current findings are consistent with the existing literature that emphasizes the capacity of selenium

for detoxification in the context of heavy metal toxicity. This protective effect is mainly attributed to the upregulation of antioxidant defenses facilitated by selenoproteins, such as glutathione peroxidase and thioredoxin reductase (Zhang et al. 2013, Liu et al. 2017). Nevertheless, the inability of even higher SeMet doses to fully restore DNA integrity implies that Cd-induced nephrotoxicity may involve multifaceted pathological pathways extending beyond oxidative stress. These may include compromised DNA repair mechanisms, mitochondrial dysfunction, and pro-inflammatory responses (Haberland 2023).

Peripheral Erythrocyte (Blood) DNA Damage

Peripheral erythrocytes of *C. carpio* exhibited a significant genotoxic response to $CdCl_2$ exposure, with both the percentage of DNA-damaged cells and GDI increasing progressively with time and higher Cd concentrations ($p < 0.001$ for both parameters across time and dose levels). Similarly, exposure to varying concentrations of As resulted in a significant increase in DNA damage in the blood cells of *Oreochromis mossambicus* compared to the control group (Ahmed et al. 2011). These findings correspond with earlier reports highlighting the high sensitivity of fish erythrocytes to heavy metal-induced genotoxicity due to their nucleated nature and continuous exposure to circulating toxicants (Witeska et al. 2023).

Across all exposure durations, the 1.56 ppm Cd group consistently exhibited the highest DNA damage and GDI values, peaking at 36.3% DNA damage and 0.422 GDI at 120 h ($p < 0.001$ compared to the control and other treatment groups) (Fig. 4). This persistent elevation underscores the cumulative and enduring genotoxic effects of Cd on erythrocytes, likely driven by its inhibition of DNA repair enzymes and its capacity to generate reactive oxygen species (ROS), which cause oxidative DNA damage (Kumar et al. 2024). These results reinforce the effectiveness of comet assay-based parameters as reliable biomarkers of genotoxic stress in fish.

The protective effect of SeMet was evident, particularly in the co-exposure groups. Co-treatment with SeMet

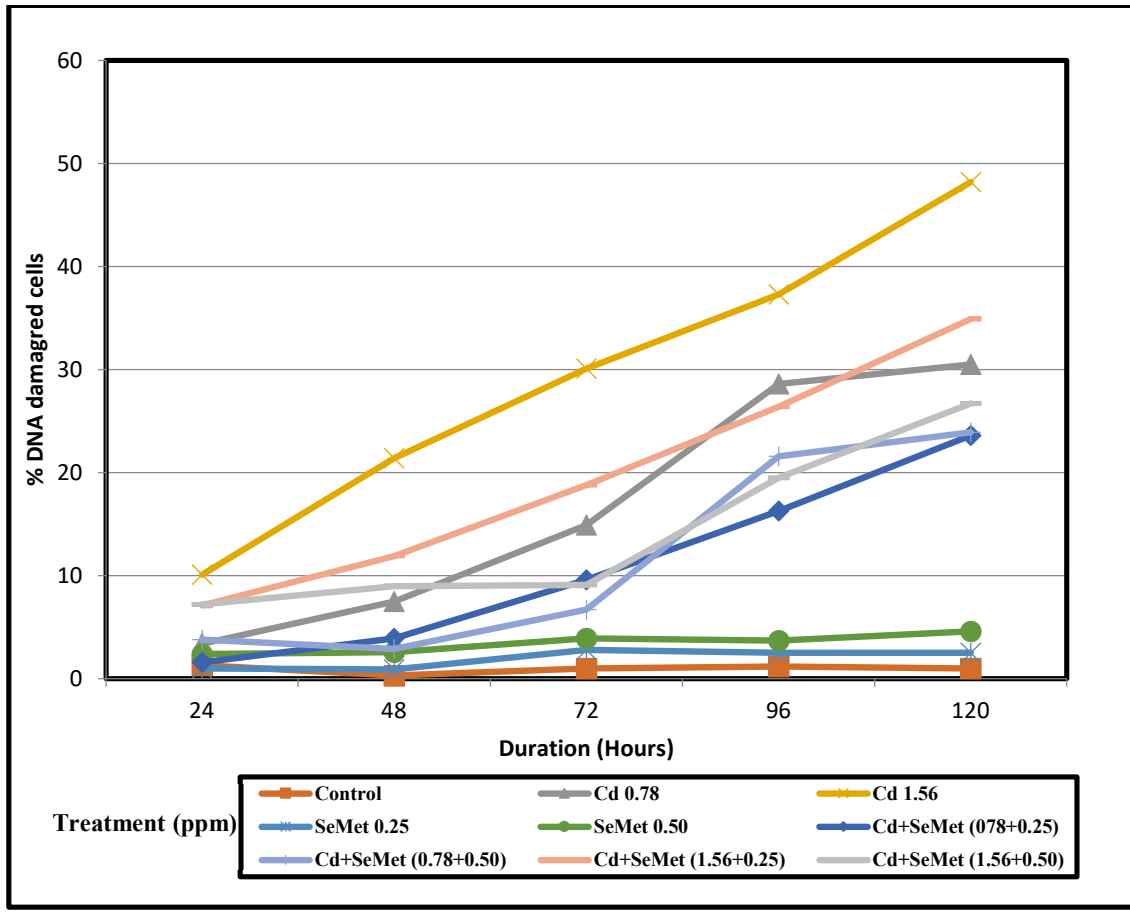

Fig. 3: Percentage of DNA damage in the kidney of *C. carpio*.

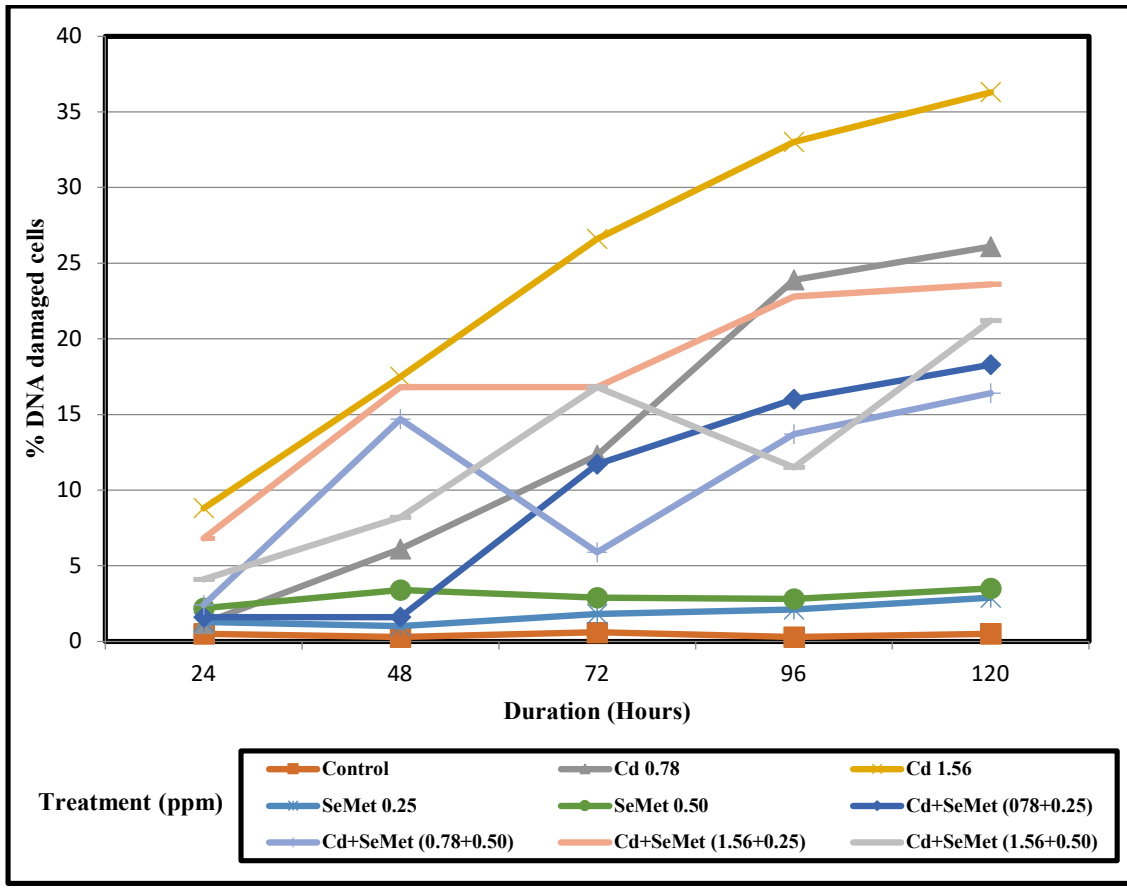

Fig. 4: Percentage of DNA damage in the blood of *C. carpio*.

significantly reduced both DNA damage percentage and GDI at all time points compared to cadmium-only groups ($p < 0.01$), suggesting that SeMet, known for its antioxidant properties, mitigates cadmium-induced oxidative stress by scavenging ROS and enhancing antioxidant defence mechanisms such as glutathione peroxidase activity (Ibrahim et al. 2024). For instance, at 120 h, co-treatment with SeMet (1.56+0.50 ppm) reduced the GDI from 0.422 to 0.248 (Fig. 4), indicating substantial mitigation of genotoxic effects.

Interestingly, SeMet at 0.50 ppm alone showed a slightly elevated GDI at 24 h (0.171), possibly reflecting a dose-dependent biphasic or hormetic response, where excess selenium may exert mild pro-oxidant effects under certain conditions ($p < 0.05$ compared to control). Such behavior has been reported in fish and other organisms, underscoring the importance of careful dose optimization when using selenium as a protective agent. Peripheral erythrocytes of *C. carpio* thus serve as reliable early indicators of systemic genotoxic stress following Cd exposure. Despite the observed protective effects, SeMet co-treatment did not fully restore DNA integrity to control levels ($p < 0.001$), indicating that while selenium supplementation is beneficial, it cannot entirely counteract Cd-induced multifactorial genotoxicity. This partial protection may be attributed to the diverse mechanisms of Cd toxicity, including interference with DNA synthesis, induction of apoptosis, and disruption of metal ion homeostasis.

The results suggest that selenium, particularly at higher concentrations, has a protective role in mitigating the genotoxic effects of Cd exposure in various tissues. The antioxidant properties of selenium are likely key to its protective effect, reducing the oxidative stress caused by Cd and thereby lowering DNA damage. The tissue-specific effects of SeMet indicate that while it offers substantial protection in some tissues (e.g., kidneys and gills), it may not fully restore DNA integrity to baseline levels in highly vulnerable tissues, such as the liver. However, its role in reducing genotoxicity in all tissues highlights its potential as a protective agent against heavy metal-induced toxicity. This offers a promising avenue for future research and potential solutions for Cd exposure.

The order of tissue sensitivity based on genotoxic damage was as follows: liver > kidney > gill > erythrocytes. Based on the metal pollution index, the livers and kidneys, followed by the gills, showed the maximum overall metal load. The degree of DNA damage (assessed by comet and diphenylamine assays) was relative to the accumulated metals in tissues, with species and site specification. Ahmed et al. (2011) reported that exposure to lead chloride resulted in the highest level of DNA damage in the liver tissue of the freshwater fish *Anabas testudineus*, followed by the kidney and gill tissues. Overall, the results suggest that selenium, particularly at higher concentrations, plays a crucial protective role in mitigating the genotoxic effects of Cd exposure in various tissues. Its antioxidant properties are likely key to its protective effect, reducing the oxidative stress caused by Cd and thereby lowering DNA damage. The tissue-specific effects of SeMet indicate that while it offers substantial protection in some tissues (e.g., kidney and gills), it may not fully restore DNA integrity to baseline levels in highly vulnerable tissues such as the liver. However, its role in reducing genotoxicity across all tissues highlights its potential as a protective agent against heavy metal-induced toxicity.

The differential sensitivity of various tissues to Cd, coupled with the protective efficacy of SeMet, has significant implications for environmental biomonitoring. The kidneys and liver serve as sensitive biomarkers for assessing heavy metal toxicity, whereas blood provides a practical and non-lethal option for routine genotoxic screening. In this regard, the comet assay is a robust and reliable early warning tool for detecting sublethal exposure to pollutants in aquatic organisms. This study emphasizes the potential of SeMet as an effective chemoprotective agent in aquatic toxicology. By substantially mitigating Cd-induced genotoxicity without inflicting harm, SeMet represents a promising strategy for implementation in aquaculture and environmental remediation, aimed at reducing the ecological consequences of heavy metal pollution in freshwater ecosystems.

CONCLUSIONS

This study demonstrated that $CdCl_2$ induced significant dose- and time-dependent DNA damage in *C. carpio*. The kidney and liver show the highest sensitivity owing to their detoxification and metal accumulation roles. However, the effective mitigation of Cd-induced genotoxicity by

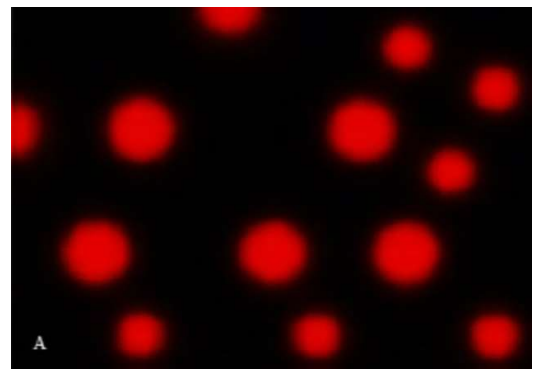

Fig. 5A: Control group of *C. carpio* showing no DNA damage nucleoids.

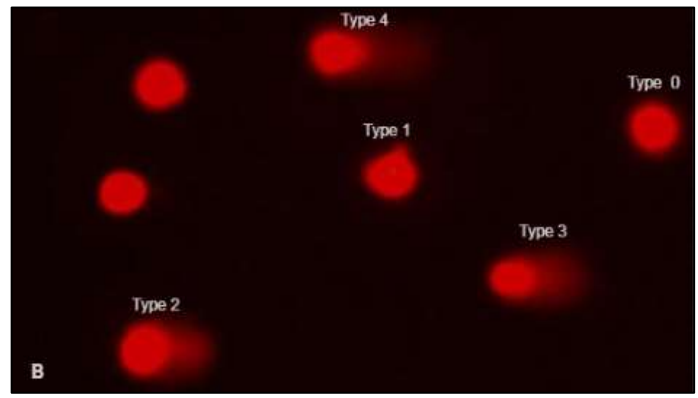

Fig. 5B: *C. carpio* exposed to cadmium chloride showing all five types of DNA damage nucleoids.

SeMet underscores its potential as a chemoprotective agent in aquatic toxicology and brings hope for the future of environmental management.

Given *C. carpio*'s importance as a food species, cadmium contamination poses ecological and public health risks. Therefore, future research should investigate the combined effects of pollutants, life stage variability, and sex-specific responses. These areas of study will provide a more comprehensive understanding of the full biological impact of Cd. Furthermore, these findings strongly advocate the application of SeMet in aquaculture and environmental management to reduce heavy metal toxicity and strengthen ecosystem resilience. The incorporation of oxidative stress biomarkers or antioxidant enzyme activity in the future would substantiate the antioxidant-based protective role of SeMet.

REFERENCES

Ahmed, M.K., Habibullah-Al-Mamun, M., Hossain, M.A., Arif, M., Parvin, E., Akter, M.S. and Islam, M.M., 2011. Assessing the genotoxic potentials of arsenic in tilapia (Oreochromis mossambicus) using alkaline comet assay and micronucleus test. *Chemosphere*, 84(1), pp.143-149.

Angelone, T., Rocca, C., Lionetti, V., Penna, C. and Pagliaro, P., 2024. Expanding the frontiers of guardian antioxidant selenoproteins in cardiovascular pathophysiology. *Antioxidants and Redox Signalling*, 40(7-9), pp.369-432.

Ardeshir, R.A., Movahedinia, A.-A. and Rastgar, S., 2017. Fish liver biomarkers for heavy metal pollution: a review article. *American Journal of Toxicology*, 2(1), pp.1-8.

Bajpayee, M., Kumar, A. and Dhawan, A., 2016. The comet assay: a versatile tool for assessing DNA damage. *Ebook Collection, Issues in Toxicology*, 1(1), pp.1-64.

Carolin, C.F., Kumar, P.S., Saravanan, A., Joshiba, G.J. and Naushad, M., 2017. Efficient techniques for the removal of toxic heavy metals from the aquatic environment: a review. *Journal of Environmental Chemical Engineering*, 5(3), pp.2782-2799.

Collins, A., Møller, P., Gajski, G., Vodenková, S., Abdulwahed, A. anderson, D. and Azqueta, A., 2023. Measuring DNA modifications with the comet assay: a compendium of protocols. *Nature Protocols*, 18(3), pp.929-989.

Company, R., Serafim, A., Cosson, R.P., Fiala-Médioni, A., Camus, L., Serrão-Santos, R. and Bebianno, M.J., 2010. Sub-lethal effects of cadmium on the antioxidant defence system of the hydrothermal vent mussel Bathymodiolus azoricus. *Ecotoxicology and Environmental Safety*, 73(5), pp.788-795.

Cuypers, A., Plusquin, M., Remans, T., Jozefczak, M., Keunen, E., Gielen, H. and Smeets, K., 2010. Cadmium stress: an oxidative challenge. *Biometals*, 23(6), pp.927-940.

Das, S., Kar, I. and Patra, A.K., 2023. Cadmium induced bioaccumulation, histopathology, gene regulation in fish and its amelioration–a review. *Journal of Trace Elements in Medicine and Biology*, 79(1), pp.127202.

Elia, A.C., Prearo, M., Pacini, N., Dörr, A.J.M. and Abete, M.C., 2011. Effects of selenium diets on growth, accumulation and antioxidant response in juvenile carp. *Ecotoxicology and Environmental Safety*, 74(2), pp.166-173.

Farhangi, M. and Jafaryan, H., 2019. The comparison of acute toxicity (96h) of copper (CuSO4) in Cyprinus carpio and Rutilus rutilus. *Environmental Pollution*, 8(2), pp.21-30.

Ferro, J.P., Ferrari, L. and Eissa, B.L., 2021. Acute toxicity of cadmium to freshwater fishes and its relationship with body size and respiratory strategy. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 248(1), pp.109109.

Forouhar Vajargah, M., Mohamadi Yalsuyi, A., Hedayati, A. and Faggio, C., 2018. Histopathological lesions and toxicity in common carp (Cyprinus carpio L. 1758) induced by copper nanoparticles. *Microscopy Research and Techniques*, 81(7), pp.724-729.

García-Medina, S., Galar-Martínez, M., Cano-Viveros, S., Ruiz-Lara, K., Gómez-Oliván, L.M., Islas-Flores, H. and Chanona-Pérez, J.J., 2022. Bioaccumulation and oxidative stress caused by aluminium nanoparticles and the integrated biomarker responses in the common carp (Cyprinus carpio). *Chemosphere*, 288(1), pp.132462.

Genchi, G., Sinicropi, M.S., Lauria, G., Carocci, A. and Catalano, A., 2020. The effects of cadmium toxicity. *International Journal of Environmental Research and Public Health*, 17(11), pp.3782.

Ghosh, K. and Indra, N., 2018. Cadmium treatment induces echinocytosis, DNA damage, inflammation, and apoptosis in cardiac tissue of albino Wistar rats. *Environmental Toxicology and Pharmacology*, 59(1), pp.43-52.

Grover, P., Danadevi, K., Mahboob, M., Rozati, R., Banu, B.S. and Rahman, M.F., 2003. Evaluation of genetic damage in workers employed in pesticide production utilizing the comet assay. *Mutagenesis*, 18(2), pp.201-205.

Haberland, V.M., Magin, S., Iliakis, G. and Hartwig, A., 2023. Impact of manganese and chromate on specific DNA double-strand break repair pathways. *International Journal of Molecular Sciences*, 24(12), pp.10392.

Hakem, R., 2008. DNA-damage repair, the good, the bad, and the ugly. *The EMBO Journal*, 27(4), pp.589-605.

Hashtjin, Y.A., Raeeszadeh, M. and Khanghah, A.P., 2024. Interaction of heavy metals (cadmium and selenium) in an experimental study on goldfish: hematobiochemical changes and oxidative stress. *Journal of Xenobiotics*, 15(2), pp.57.

Hayat, M.T., Nauman, M., Nazir, N., Ali, S. and Bangash, N., 2019. Environmental hazards of cadmium: past, present, and future. In: *Cadmium Toxicity and Tolerance in Plants*, pp.163-183. Academic Press.

Husen, M.A. and Sharma, S., 2015. Anaesthetics efficacy of MS-222, Benzoak® vet, AQUI-S® and clove oil on common carp (Cyprinus carpio) fry. *International Journal of Research in Fisheries and Aquaculture*, 5(3), pp.104-114.

Ibrahim, D., Pet, I., Anter, R.G., Abdelwarith, A.A., Rahman, M.M.I.A., Shafik, B.M. and Kishawy, A.T., 2024. Marine Smenospongia extract mitigated co-infection with Trichodina sp. and Flavobacterium columnare in Nile tilapia: insights into promoting growth performance, immune, antioxidant and autophagy defenses, and suppression of endoplasmic reticulum stress-related genes. *Frontiers in Marine Science*, 11(1), pp.1475150.

International Agency for Research on Cancer (IARC), 1993. Cadmium and cadmium compounds. *Monographs on Evaluation of Carcinogenic Risks to Humans*, 58, pp.119-237.

Irfan, M., Liu, X., Hussain, K., Mushtaq, S., Cabrera, J. and Zhang, P., 2021. The global research trend on cadmium in freshwater: a bibliometric review. *Environmental Science and Pollution Research*, 28(1), pp.1-14.

Javed, M., Ahmad, I., Usmani, N. and Ahmad, M., 2016. Studies on biomarkers of oxidative stress and associated genotoxicity and histopathology in Channa punctatus from heavy metal polluted canal. *Chemosphere*, 151(1), pp. 210-219.

Jha, A.N., 2023. Measuring DNA modifications with the comet assay: a compendium of protocols. *Nature Protocols*, 18(1), pp.1-65.

Jiang, N., Naz, S., Ma, Y., Ullah, Q., Khan, M.Z., Wang, J. and Basang, W.D., 2023. An overview of comet assay application for detecting DNA damage in aquatic animals. *Agriculture*, 13(3), pp.623.

Joseph, L., Jun, B.M., Flora, J.R., Park, C.M. and Yoon, Y., 2019. Removal of heavy metals from water sources in the developing world using low-cost materials: a review. *Chemosphere*, 229(1), pp.142-159.

Khan, Z., Elahi, A., Bukhari, D.A. and Rehman, A., 2022. Cadmium sources, toxicity, resistance and removal by microorganisms- A potential strategy for cadmium eradication. *Journal of Saudi Chemical Society*, 26(6), pp.101569.

Kieliszek, M. and Błażejak, S., 2013. Selenium: significance and outlook for supplementation. *Nutrition*, 29(5), pp.713-718.

Klobučar, G.I., Malev, O., Šrut, M., Štambuk, A., Lorenzon, S., Cvetković, Ž. and Maguire, I., 2012. Genotoxicity monitoring of freshwater environments using caged crayfish (Astacus leptodactylus). *Chemosphere*, 87(1), pp.62-67.

Kora, A.J., 2018. Bacillus cereus, selenite-reducing bacterium from contaminated lake of an industrial area: a renewable nanofactory for the synthesis of selenium nanoparticles. *Bioresource Bioprocess*, 5(1), pp.1-12.

Kubier, A., Wilkin, R.T. and Pichler, T., 2019. Cadmium in soils and groundwater: a review. *Applied Geochemistry*, 108(1), pp.104388.

Kumar, R., Nagpure, N.S., Kushwaha, B., Srivastava, S.K. and Lakra, W.S., 2010. Investigation of the genotoxicity of malathion to freshwater teleost fish Channa punctatus (Bloch) using the micronucleus test and comet assay. *Archives of Environmental Contamination and Toxicology*, 58(2), pp.123-130.

Kumar, U., Jha, A.K. and Kumar, N., 2024. Cadmium toxicity in the environment: sources, issues, remediation, and challenges. In: *Cadmium Toxicity: Challenges and Solutions*, pp.1-28. Cham: Springer Nature Switzerland.

Li, Z.M., Wang, X.L., Jin, X.M., Huang, J.Q. and Wang, L.S., 2023. The effect of selenium on antioxidant system in aquaculture animals. *Frontiers in Physiology*, 14(1), pp.1153511.

Liao, G., Wang, P., Zhu, J., Weng, X., Lin, S., Huang, J. and Meng, X., 2021. Joint toxicity of lead and cadmium on the behavior of zebrafish larvae: an antagonism. *Aquatic Toxicology*, 238(1), pp.105912.

Liu, G.X., Jiang, G.Z., Lu, K.L., Li, X.F., Zhou, M., Zhang, D.D. and co-authors, 2017. Effects of dietary selenium on the growth, selenium status, antioxidant activities, muscle composition and meat quality of blunt snout bream, Megalobrama amblycephala. *Aquaculture Nutrition*, 23(6), pp.777-787.

Mancera-Rodríguez, N.J., Galiano, D.R., López-Montoya, A.J., Llorent-Martínez, E.J., Molina-García, L. and Azorit, C., 2024. Common carp as an ecological indicator of environmental pollution in reservoirs of southern Spain: inferring the environmental risks of anthropogenic activities. *Environmental Science and Pollution Research*, 31(25), pp.36192-36206.

Marieke, S., 2020. L-selenomethionine: a powerful antioxidant for commercial fish culture trials. *Aquafeed (ORFFA): Advances in Processing & Formulation*, 12(3), pp.49-52.

McKelvey, S.M., Horgan, K.A. and Murphy, R.A., 2015. Chemical form of selenium differentially influences DNA repair pathways following exposure to lead nitrate. *Journal of Trace Elements in Medicine and Biology*, 29(1), pp.151-169.

Mechlaoui, M., Dominguez, D., Robaina, L., Geraert, P.A., Kaushik, S., Saleh, R. and Izquierdo, M., 2019. Effects of different dietary selenium sources on growth performance, liver and muscle composition, antioxidant status, stress response and expression of related genes in gilthead seabream (Sparus aurata). *Aquaculture*, 507(1), pp.251-259.

Mielcarek, K., Nowakowski, P., Puścion-Jakubik, A., Gromkowska-Kępka, K.J., Soroczyńska, J., Markiewicz-Żukowska, R. and Socha, K., 2022. Arsenic, cadmium, lead and mercury content and health risk assessment of consuming freshwater fish with elements of chemometric analysis. *Food Chemistry*, 379(1), pp.132167.

Minarik, T.A., Vick, J.A., Schultz, M.M., Bartell, S.E., Martinovic-Weigelt, D., Rearick, D.C. and Schoenfuss, H.L., 2014. On-site exposure to treated wastewater effluent has subtle effects on male fathead minnows and pronounced effects on carp. *Journal of the American Water Resources Association*, 50(2), pp.358-375.

Niu, Y., Jiang, X., Wang, K., Xia, J., Jiao, W., Niu, Y. and Yu, H., 2020. Meta-analysis of heavy metal pollution and sources in surface sediments of Lake Taihu, China. *Science of the Total Environment*, 700(1), pp.134509.

Noor, Z., Khan, S.A. and Noor, M., 2020. Assessment of cadmium toxicity and its possible effects on goldfish (Carassius auratus), employing microscopy and biochemical techniques. *Microscopy Research and Techniques*, 83(12), pp.1441-1449.

Obaiah, J., Vivek, C., Padmaja, B., Sridhar, D. and Peera, K., 2020. Cadmium toxicity impact on aquatic organisms-oxidative stress: implications for human health, safety and environmental aspects: a review. *International Journal of Scientific Research*, 9(1), pp.4172-4185.

Palaniappan, V. and Karthikeyan, K., 2022. Potassium permanganate: a 'desert island drug' in dermatology. *Clinical and Experimental Dermatology*, 47(9), pp.1650-1657.

Pandey, A.K., Nagpure, N.S., Trivedi, S.P., Kumar, R. and Kushwaha, B., 2011. Profenofos induced DNA damage in freshwater fish, Channa punctatus (Bloch) using alkaline single cell gel electrophoresis. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 726(2), pp.209-214.

Qiao, D., Wang, G., Li, X., Wang, S. and Zhao, Y., 2020. Pollution, sources and environmental risk assessment of heavy metals in the surface AMD water, sediments and surface soils around unexploited Rona Cu deposit, Tibet, China. *Chemosphere*, 248(1), pp.125988.

Qu, F. and Zheng, W., 2024. Cadmium exposure: mechanisms and pathways of toxicity and implications for human health. *Toxics*, 12(6), pp.388.

Rahoui, S., Ben, C., Chaoui, A., Martinez, Y., Yamchi, A., Rickauer, M. and El Ferjani, E., 2014. Oxidative injury and antioxidant genes regulation in cadmium-exposed radicles of six contrasted Medicago truncatula genotypes. *Environmental Science and Pollution Research*, 21(13), pp.8070-8083.

Saravanan, P., Saravanan, V., Rajeshkannan, R., Arnica, G., Rajasimman, M., Gurunathan, B. and Pugazhendhi, A., 2024. Comprehensive review on toxic heavy metals in the aquatic system: sources, identification, treatment strategies, and health risk assessment. *Environmental Research*, 234(1), pp.119440.

Sharma, M., Kant, R., Sharma, A.K. and Sharma, A.K., 2024. Exploring the impact of heavy metals toxicity in the aquatic ecosystem. *International Journal of Energy and Water Resources*, 8(1), pp.1-14.

Singh, N.P., McCoy, M.T., Tice, R.R. and Schneider, E.L., 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental Cell Research*, 175(1), pp.184-191.

Singh, R., Gautam, N., Mishra, A. and Gupta, R., 2011. Heavy metals and living systems: an overview. *Indian Journal of Pharmacology*, 43(3), pp.246-253.

Ta, T.Y., Le, T.T., Trinh, T.T., Trinh, T.T., Pham, T.M.T. and Pham, T.H.P., 2018. Risk assessment of lead and cadmium on juveniles of Cyprinus carpio in laboratory scale. *Vietnam Journal of Science, Technology and Engineering*, 60(2), pp.78-83.

Taysi, M.R., 2024. Assessing the effects of cadmium on antioxidant enzymes and histological structures in rainbow trout liver and kidney. *Scientific Reports*, 14(1), pp.27453.

Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K. and Sutton, D.J., 2012. Heavy metal toxicity and the environment. *Molecular, Clinical and Environmental Toxicology: Volume 3: Environmental Toxicology*, pp.133-164.

Thévenod, F., 2009. Cadmium and cellular signaling cascades: to be or not to be? *Toxicology and Applied Pharmacology*, 238(3), pp.221-239.

Vardhan, K.H., Kumar, P.S. and Panda, R.C., 2019. A review on heavy metal pollution, toxicity and remedial measures: current trends and future perspectives. *Journal of Molecular Liquids*, 290(1), pp.111197.

Wande, Y., Jie, L., Aikai, Z., Yaguo, Z., Linlin, Z., Yue, G., et al. 2020. Berberine alleviates pulmonary hypertension through Trx1 and β-catenin signaling pathways in pulmonary artery smooth muscle cells. *Experimental Cell Research*, 390(1), pp.111910.

Wang, Q.E., 2015. DNA damage responses in cancer stem cells: implications for cancer therapeutic strategies. *World Journal of Biological Chemistry*, 6(3), pp.57.

Wang, Y., Yan, Q., Shi, Y. and Long, M., 2024. Copper toxicity in animals: a review. *Biological Trace Element Research*, pp.1-12.

Wenfi, J., 2023. The role of selenium as a bioactive antioxidant. *Oxidants and Antioxidants in Medical Science*, 12(8), pp.01-02.

Witeska, M., Kondera, E. and Bojarski, B., 2023. Hematological and hematopoietic analysis in fish toxicology- A review. *Animals*, 13(16), pp. 2625.

Wu, X., Cobbina, S.J., Mao, G., Xu, H., Zhang, Z. and Yang, L., 2016. A review of toxicity and mechanisms of individual and mixtures of heavy metals in the environment. *Environmental Science and Pollution Research*, 23(9), pp.8244-8259.

Yancheva, V., Stoyanova, S., Todorova, B., Georgieva, E. and Velcheva, I., 2022. Common carp (Cyprinus carpio Linnaeus, 1785): a species equally important for aquaculture and aquatic toxicology. *ZooNotes*, 199(1), pp.1-3.

Zhang, M., An, C., Gao, Y., Leak, R.K., Chen, J. and Zhang, F., 2013. Emerging roles of Nrf2 and phase II antioxidant enzymes in neuroprotection. *Progress in Neurobiology*, 100(1), pp.30-47.

Zheng, J.L., Peng, L.B., Xia, L.P., Li, J. and Zhu, Q.L., 2021. Effects of continuous and intermittent cadmium exposure on HPGL axis, GH/IGF axis and circadian rhythm signaling and their consequences on reproduction in female zebrafish: biomarkers independent of exposure regimes. *Chemosphere*, 282(1), pp.130-879.

Zuo, H., Yuan, J., Yang, L., Liang, Z., Weng, S. and He, J., 2019. Characterization and immune function of the thioredoxin-interacting protein (TXNIP) from Litopenaeus vannamei. *Fish & Shellfish Immunology*, 84(1), pp.20-27.