Proceedings of the 5th International Conference on Advanced Science & Engineering ICOASE 2025, University of Zakho and Duhok Polytechnic University, 24th – 25th September 2025

(Special issue for Passer Journal of Basic and Applied Sciences)
Paper No. 14

Quercus Infectoria Counteracts Lead Toxicity in Carp: Growth, Metabolic & Enzymatic Protection

Payman Mohammed Saleem Abdulrahman [1], Basim. A. S. Al Sulivany [2], Muhammad Owais [2], Oyegoke Rukayat Abiodun [3]

[1] Department of Biology, College of Science, University of Zakho, Zakho 42002, Duhok, Kurdistan Region, Iraq.
[2] Department of Zoology, Emerson University 60000, Multan, Punjab, Pakistan.
[3] Department of Biochemistry, Faculty of Life Sciences, University of Ilorin 240003, P. M. B. 1515. Ilorin, Nigeria.
Email: paymanmohammad6@gmail.com , basim.ahmed@uoz.edu.krd , owaisgulmuhammad@gmail.com , and oyegoke.ra@unilorin.edu.ng

Abstract

The research project aims to determine the protective effects of *Quercus infectoria* seeds (QIS) on lead nitrate ($Pb(NO_3)_2$) induced toxicity in *Cyprinus carpio* (common carp). Eighty juvenile carp weighted (151.4 ± 6.4 g) were divided into four groups: T0 (control), fed standard diet; the fish in the second group T1, exposed to 5 mg/L of $Pb(NO_3)_2$; T2, were fed a QIS-supplemented diet (10 g/kg); and T3, were exposed to $Pb(NO_3)_2$ + QIS. the parameters of the growth were severely influenced in T1, with final weight (171.1±4.748g) significantly lower than those present in the T0 (235.4±11.6g; $p< 0.0001$). On the other hand, QIS supplementation (T2) showed the highest growth (257.4±12.73g), as compared with both control and Pb-exposed groups ($p< 0.0001$). The T3 group demonstrated intermediate growth (223±12.36g), indicating QIS partially counteracted Pb toxicity. Biochemical analysis revealed that Pb-exposed fish (T1) developed hyperglycemia (77.37±4.628mg/dL when compared with T0: 65.4±1.65 mg/dL) and elevated urea (5.3±0.55mg/dL) as compared with the T0 group (3.733±0.66 mg/dL). In contrast, QIS groups showed improved metabolic profiles. Tissue enzyme activities (alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP)) in muscle and spleen confirmed Pb-induced damage, with QIS significantly reducing oxidative stress markers ($p< 0.05$). These results conclude that QIS effectively reduced the toxicity of Pb by elevating the growth parameters, protecting the tissue, and supporting its use as a natural feed additive in aquaculture.

Keywords: C. carpio, Growth Performance, Biochemistry, Enzyme activity, Lead poisoning.

1 Introduction

Aquaculture has steadily grown into a vital sector for food production, significantly and enhancing the local and international economies[1,2]. Fish are known for their rich nutritional profile they are best sources of high protein quality, essential fatty acids, vitamins, and minerals [3,4]. However, the rapid expansion of aquaculture has raised questions around its environmental footprint and the safety of its products, highlighting the need for more sustainable and regulated practices [5,6]. Fish, the most diverse vertebrate group, are found in nearly every type of aquatic environment [7]. This diversity makes them crucial for the global food chain and incredibly useful in scientific studies on evolution, ecology, and physiology [8]. Their adaptability to various environments and economic and nutritional value has made them indispensable to researchers and consumers [9]. On the nutritional front, fish offer a wide range of health benefits. They are packed with omega-3 fatty acids, all essential amino acids, and several beneficial bioactive compounds [10]. Beyond that, fish are rich in important micronutrients such as vitamin D, B vitamins, iron, zinc, and selenium. These nutrients support critical bodily functions and protect against various diseases [11]. Regular fish consumption has been linked to a decreased risk of chronic conditions like heart disease, certain cancers, and diabetes [12]. Despite these advantages, aquaculture faces ongoing challenges, particularly due to environmental pollution. Toxic substances like arsenic and cadmium can enter aquatic ecosystems and accumulate in fish tissues, affecting their health and, eventually, that of humans who consume them [13, 14]. Such contamination can impair fish growth, reproduction, and physiological stability, with broader ecological consequences [15,16]. These risks make it critical to enforce stronger environmental protections [17,18].

Water-based environments worldwide are facing growing pressure from human-made contamination. Among the most persistent and harmful pollutants are heavy metals, which build up in living organisms and remain hazardous for long periods [19]. Among these, lead (Pb) is particularly concerning. It enters water from various sources such as industrial discharge, runoff from pesticide-laden agricultural lands, and untreated or poorly treated urban sewage [20]. Once introduced into aquatic systems, Pb can move up the food chain, often impacting key aquaculture species like *Cyprinus carpio* (*C. carpio*; common carp), which makes up nearly 10% of the world's freshwater aquaculture output [21]. Carps are notably sensitive to Pb exposure because the metal accumulates in tissues with high metabolic activity. Studies have consistently shown that Pb builds up in the liver first, followed by the gills, spleen, kidneys, and muscle tissue [22, 23]. This accumulation pattern is closely linked with physiological disturbances, including impaired osmoregulation, suppression of antioxidant defense mechanisms, and disruption in blood cell formation [24]. Experimental data from long-term exposure studies suggest that lead concentrations between 0.5 and 2.0 mg/L can lead to significant reductions in growth (by 18–32%) and reproductive success (by 40–60%) in affected fish [25].

Lead nitrate ($Pb(NO_3)_2$), a toxic heavy metal compound, interferes with normal metabolic functions in fish, often leading to oxidative damage, disruptions in blood parameters, and weakened immune defenses [26,27]. Prolonged exposure to this contaminant has been associated with slowed growth, imbalanced enzyme activity in vital organs like the liver and spleen, and a general decline in fish health [28]. Considering the long-lasting presence of lead in aquatic systems, finding effective and sustainable methods to counteract its toxic effects is becoming increasingly important for modern aquaculture [29]. Natural remedies derived from medicinal plants have recently gained attention for their detoxifying properties. One such plant, *Quercus infectoria (Q. infectoria*; QIS*)*, has shown considerable promise due to its abundance of bioactive compounds such as tannins, flavonoids, and polyphenols [30]. These substances are known for supporting liver function, stimulating immune responses, and reducing inflammation, offering a natural defense against heavy metal toxicity [31]. Integrating QIS into fish diets has demonstrated improved growth, better blood health, and normalized enzyme activity in fish exposed to environmental contaminants [32].

This research evaluates how *C. carpio* responds to lead nitrate exposure and dietary supplementation with *Q. infectoria*. The study assesses growth rates, biochemical markers, and enzyme functions in muscle and spleen tissues. The goal is to determine whether QIS can provide a protective effect and support healthier fish under toxic conditions, paving the way for safer, more resilient aquaculture practices.

2 Materials and Methods

2. 1 Ethical Approval and Consent

All animal procedures followed ethical standards and were approved by the University of Zakho's College of Science Animal Ethics Committee (AEC-058; 2024).

2. 2 Fish collecting and study area

Eighty juvenile common carp were obtained from the special commercial hatchery in Khanke township, Summil district, Duhok, Iraq. The fish's average body weight was 151.4 ± 6.4 g. Khanke lies near the Mosul Dam (Chambarakat Dam in Kurdish language), about seventy kilometers (km) south of Duhok and fifty km north of Mosul, along the Tigris River [33,34].

Following collection, the fish were transported to the Laboratory of Fish, Department of Biology, College of Science, University of Zakho, the fish were kept under standard laboratory conditions for 15 days. The fish were maintained in circular polyethylene tanks measuring 100 × 71.4 cm, with a capacity of approximately 400 liters. To reduce the external pathogens, the carp were briefly immersed (1–2 minutes) in a saline solution of sodium chloride purchased from Sigma-Aldrich [35,36]. The fish were fed a commercial basal diet purchased from the Amedi Company for Animal Feed.

2. 3 Diet preparation

The plant seeds of *Q. infectoria* (QIS) were collected from the Batifa mountains in Zakho. The seeds were grinded into a powder and thoroughly blended with the other feed ingredients using an electric mixer. The resulting mixture was then mixed with water to form a smooth dough. This dough was processed through a homemade pasta machine with an electric extruder and a 2 mm mesh plate to form uniform pellets. The freshly extruded pellets were dried overnight at 50°C and then stored at -18°C until use [37,38]. The diet ingredient details are presented in Table 1.

Table 1: Ingredients of experimental feed for *C. carpio.*

Ingredient	T0 Control	T1 Pb $(NO_3)_2$*	T2 QIS	T3 $Pb(NO_3)_2$+QIS
Fish Meal	16	16	16	16
Corn	14	14	14	14
Soybean Meal	28	28	28	28
Barley	17	17	17	17
Wheat	22	22	22	22
Premix	2	2	2	2
Ascorbic acid	1	1	0.9	0.9
QIS			0.1	0.1
Total	100	100	100	100

*Lead nitrate [$Pb(NO_3)_2$] used in the experiment was sourced from Sigma-Aldrich (Turkey).

2. 4 Experimental Design

After acclimatization and feed preparation, the *C. carpio* was selected on the following basis: (a) Skin with no sign of disease, (b) no alteration in their skin, and (c) gill coloration [39,40]. The fish were randomly divided into four experimental groups, each consisting of duplicate tanks (ten fish per tank). The groups were designated as follows: T0 (Control): Fish fed only the basal diet pellet. The fish in the second group, T1, were exposed to 5 mg/L Pb $(NO_3)_2$ while fed the basal diet. In the third group, T2, the fish were fed the basal diet supplemented with 10 g/kg QIS. The fish in the last group, T3, were simultaneously exposed to 5 mg/L Pb $(NO_3)_2$ and fed the basal diet supplemented with 10 g/kg QIS. The feeding trial duration lasted for 60 days. Fish were fed once daily at a rate of 3% of their body weight. Uneaten feed and fecal residues were removed daily using a siphon to maintain water quality. Throughout the experimental period, no mortality was observed among the fish.

2. 5 Physicochemical parameters

The aeration in each tank was continuously provided using by using Chinese air compressors (Hailea ACO-318, 45 W, 70 L/min; Hailea ACO-328, 55 W, 82 L/min; Resun ACO-010, 200 W, 0.135 m^3/min). Water quality parameters, including pH, dissolved oxygen, and temperature, were monitored daily and maintained at optimal levels: pH 8.28 ± 0.23, dissolved oxygen 7.4 ± 0.4 mg/L, temperature 16 ± 0.3°C, and salinity 0.06 g/L [41,42,43].

2. 6 Growth Performance Measurement

After exposing the fish to different diet concentrations, every twenty days, the weight gain was measured for 60 days by using an electronic balance [44].

2. 7 Biochemical and Enzyme Activity Parameters

For serum biochemical parameter (glucose, urea, uric acid, total protein, alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP)) measurement, the blood was collected by using sterile syringes from the caudal vein of the fish. The collected blood samples were transferred into gel tubes, following a 30-minute clotting period at 4°C, serum was separated by centrifugation at 3,000 × g for 15 minutes for use to assay the above biochemical and enzyme activity parameters, and was carried out using COBAS C111 Automated Analyzer.

2. 8 Tissue Enzyme Activity.

After blood collection, the fish (5 per tank) were caught in a dip net and killed by a sharp blow to the head. Pieces of spleen and muscle tissue (2 g, 1×1 cm) were collected by sterile scalpel, kept in a plastic container, and stored in the freezer until used for determining ALT, AST, LDH, and ALP.

For ALT, AST, and LDH determination, the tissue (spleen and muscle) was homogenized by using (E2355 Con-Torque Tissue Homogenizer- Eberbach) in 0.15 M Tris-HCl buffer (pH 7.4) after being rinsed with cold saline solution, dried, and weighed. The homogenate was centrifuged at 10000 g for 10 minutes. Supernatant was used to determine this enzyme activity [45].

For ALP determination, 10% (w/v) tissue homogenate was prepared from spleen and muscle in ice-cold Carbonate-Bicarbonate Buffer (PH 10.5). The homogenate was centrifuged at 10000g for 10 minutes. The supernatant was used

to determine ALP activity using the Optimized method based on the DGKC (German Society of Clinical Chemistry, 1972).

Subsequently, the supernatant from the homogenized samples was examined using the slide reagent kits for this enzyme activity and a COBAS C111 Automated Analyzer.

2. 9 Data Analysis

Statistical evaluations were carried out using GraphPad Prism software (version 9). Group comparisons were assessed through a one-way ANOVA, with post hoc testing conducted using Duncan's method to identify statistically distinct means. Differences were considered significant at a *P*-value below 0.05. All data are expressed as mean values and standard errors of the mean.

3 Results

3. 1 Growth performance

The growth parameters of *C. carpio* varied significantly across the different dietary treatments, as presented in Table 2 and Figure 1. Fish fed the basal diet (T0) recorded progressive weight gain, starting at 149 ± 16.13 g and reaching 235.4 ± 11.6 g by the end of the trial ($p < 0.0001$). However, those exposed to $Pb(NO_3)_2$ (T1) experienced markedly slower growth, with final weights (171.1 ± 4.748 g) significantly lower than the control ($p < 0.05$). The group supplemented with QIS (T2) showed a clear improvement, achieving a final weight of 257.4 ± 12.73 g, which was higher than both the control and lead-exposed groups ($p < 0.0001$). Interestingly, fish receiving both QIS and $Pb(NO_3)_2$ (T3) displayed intermediate growth (223 ± 12.36 g).

Table 2: Growth performance parameters of *C. carpio* exposed to lead and *Q. infectoria* and a combination of both during 60-day intervals.

Weight parameters	T0 (Control)	T1 $Pb(NO_3)_2$	T2 (QIS)	T3 $Pb(NO_3)_2$ +QIS	P value
Initial weight (g)	149±16.13	151.4±15.4	152.6±16.72	150.7±21.64	0.453
Weight after 20 days (g)	190±17.29a	157.9±20.89b	205.4±14.65a	185.7±18.16ab	0.0213
Weight after 40 days (g)	230.7±19.04a	168.9±19.82b	237.9±13.25a	207.1±16.62ab	0.0412
Weight after 60 days (g)	235.4±11.6a	171.1±4.748b	257.4±12.73a	223±12.36ab	<0.0001

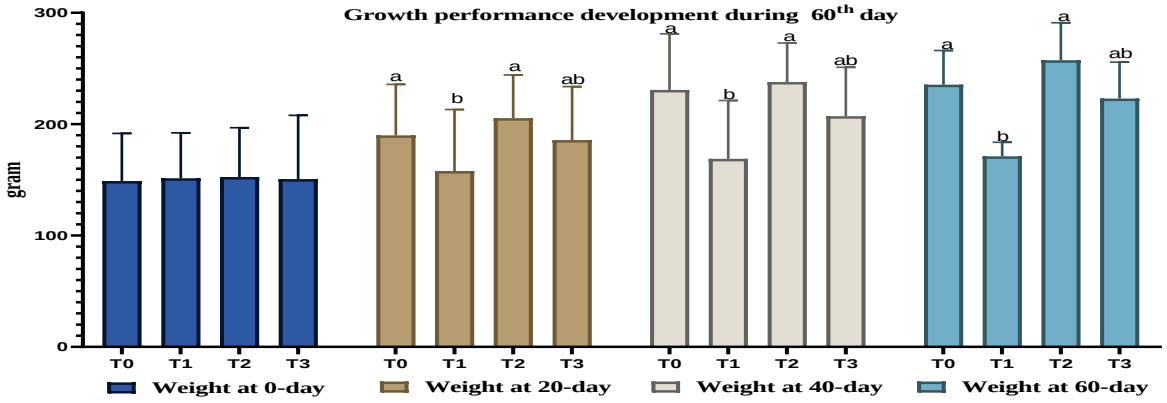

Figure 1: Growth improvement of *C. carpio* exposed to lead and *Q. infectoria* and a combination of both.

3. 2 Biochemical and Enzyme Activity

The biochemical results revealed striking differences, as Table 3 shows. The fish in the T0 group maintained stable glucose levels (65.4 ± 1.65 mg/dL), while lead-exposed fish (T1) developed significant ($p = 0.0022$) hyperglycemia (77.37 ± 4.628 mg/dL). The best statistical result came from the QIS group (T2), which showed remarkably low glucose readings (46.57 ± 2.146 mg/dL), indicating elevated glucose metabolism beyond normal levels (Figure 2; A).

When examining kidney function, the lead-exposed fish again showed distress signals. Urea concentrations elevated sharply in lead-exposed fish (5.3 ± 0.55 mg/dL compared to the T0 3.733 ± 0.66 mg/dL; p = 0.0138). However, QIS supplementation reduced urea to just (2.3 ± 1.13 mg/dL) (Figure 2; B). While uric acid levels remained stable across groups (0.93-1.15 mg/dL; p = 0.7056) (Figure 2; C), total protein content increased in QIS-fed fish at (3.63 ± 0.06 mg/dL) (Figure 2; D).

The liver enzyme profiles reveal clear findings. $Pb(NO_3)_2$ exposure (T1) caused severe hepatic stress, with ALT levels nearly doubling to 15.9 ± 4.3 U/L when compared with the fish fed basal diet pellets (8.15 ± 1.35 U/L), with a p value of 0.0105 (Figure 2; E). Similarly, AST levels were also elevated, more than doubling (228.3 ± 62.38 U/L as compared with T0 (96.95 ± 32.65 U/L; p = 0.0103) (Figure 2; F).

However, the T3 group (QIS + $Pb(NO_3)_2$) exhibited intermediate values for most parameters. Even in the dual-exposure group (T3), alkaline phosphatase levels (21 ± 2 U/L) stayed closer to normal than lead-only fish (48 ± 12.49 U/L; p = 0.0102), demonstrating QIS's partial protective effect (Figure 2; G).

Table 3: Serum Biochemical and Enzyme activity parameters of *C. carpio* exposed to lead and Q. infectoria and a combination of both during 60-day intervals.

Biochemical parameter					
	T0 (Control)	**T1 $Pb(NO_3)_2$**	**T2 (QIS)**	**T3 $Pb(NO_3)_2$ +QIS**	**P Value**
Glucose (mg/dl)	65.4±1.65a	77.37±4.628a	46.57±2.146b	69.07±5.06a	0.0022
Urea (mg/dl)	3.733±0.66a	5.3±0.55b	2.3±1.13c	3.567±0.41a	0.0138
Uric acid (mg/dl)	0.93±0.38	1.1±0.6	0.56±0.06	1.15±0.55	0.7056
Total protein (mg/dl)	3.12±0.047	2.7±0.16	3.63±0.06	2.87±0.29	0.0895
Alanine transaminase (U/l)	8.15±1.35a	15.9±4.3b	6.633±1.821a	8.133±0.42a	0.0105
Aspartate aminotransferase (U/l)	96.95±32.65a	228.3±62.38b	111.3±17.46a	201.4±6.83ab	0.0103
Alkaline phosphatase (U/l)	23.5±8.5a	48±12.49b	12.67±1.202c	21±2ac	0.0102

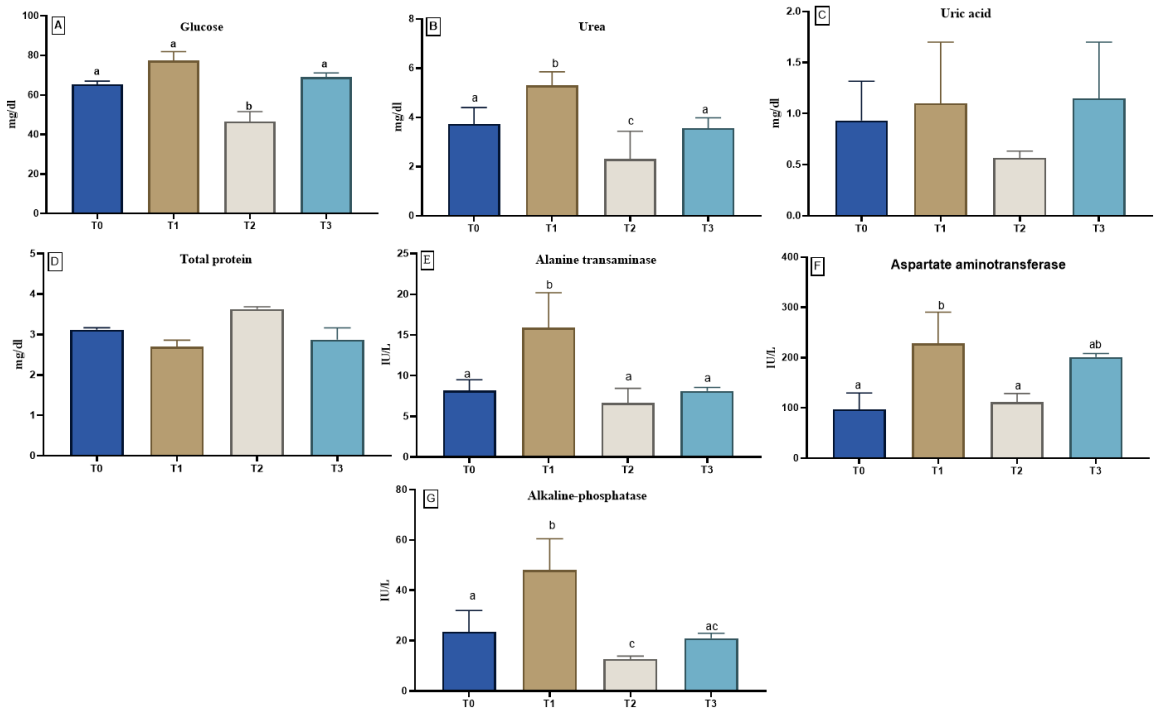

Figure 2: Reveals the biochemical and enzyme activity parameters of *C. carpio* exposed to lead and *Q. infectoria* and a combination of both during 60-day intervals.

3. 3 Muscle Enzyme Activity

The study revealed significant variations in muscle enzyme activities across different dietary treatments (T0 to T3), as indicated in Table 4 and Figure 3. The ALT levels exhibited dynamic changes, with the highest activity at T1 (85.33 ± 18.98) and the lowest at T2 (50.3 ± 4.3), showing a significant P value of 0.036. Similarly, the AST activity peaked at T1 (1500 ± 93.52) and was reduced at T2 (545 ± 272.4), with a P value of 0.0235, on the other hand, LDH demonstrated a marked increase at T1 (5739 ± 403.6) compared to T2 (1361 ± 291.5), with a P value of 0.0065. Alkaline phosphatase (ALP) activity varied significantly (P = 0.0002), with the highest level at T1 (20.67 ± 1.2) and the lowest at T2 (3.333 ± 1.202).

Table 4: Muscle enzyme activity in *C. carpio* exposed to lead and *Q. infectoria* and a combination of both during 60-day intervals.

Muscle					
Enzyme activites	**T0 (Control)**	**T1 $Pb(NO_3)_2$**	**T2 (QIS)**	**T3 $Pb(NO_3)_2$ +QIS**	**P value**
ALT (U/L)	59.15±10.75a	85.33±18.98b	50.3±4.3c	67.47±1.328ac	0.0362
AST (U/L)	726.1±220.5ab	1500±93.52b	545±272.4a	1240±100.4ab	0.0235
LDH (U/L)	2872±734.1	5739±403.6	1361±291.5	3726±853.5	0.0065
ALP (U/L)	7.667±1.856ac	20.67±1.202b	3.333±1.202a	14±1.528c	0.0002

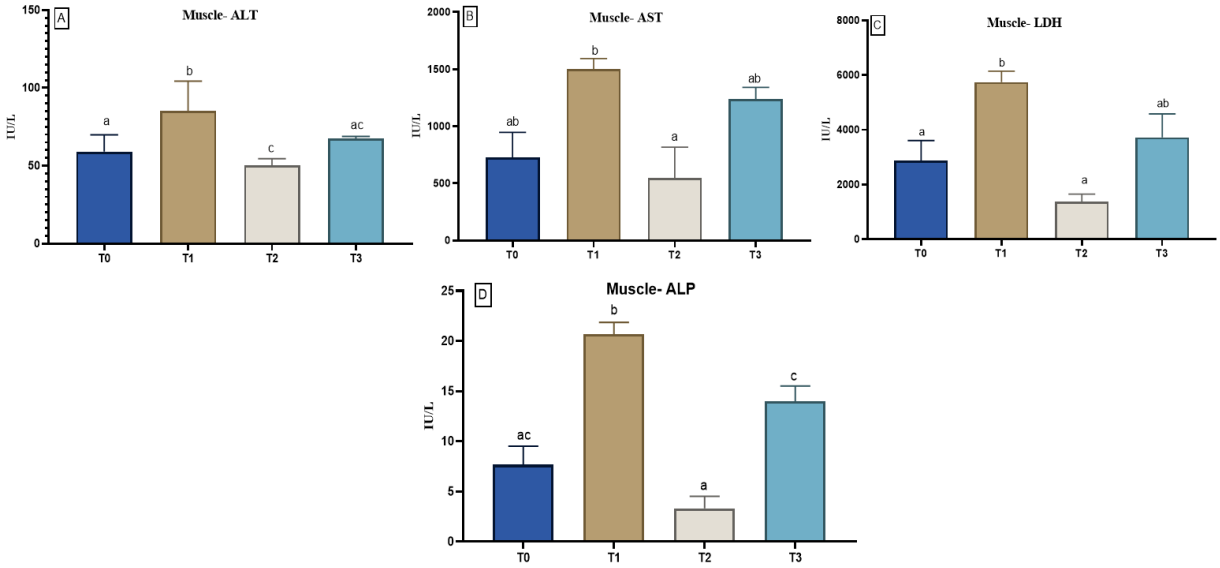

Figure 3: Reveals serum muscle enzyme activity (ALT, AST, LDH, and ALP) in *C. carpio* exposed to lead and *Q. infectoria* and a combination of both during 60-day intervals.

3. 4 Spleen Enzyme Activity

To measure the effect of dietary treatments on spleen enzyme activities. Significant variations were observed across treatment groups are presented in the Table 5 and Figure 4, the level of ALT activity increased in the lead nitrate-exposed group (116.4 ± 38.65) compared to the basal diet control (67.65 ± 2.65) and QIS-supplemented groups (65.15 ± 10.85; 76.47 ± 7.143), with a significant P value of 0.0392. Similarly, the AST levels were highest in T1 (1765 ± 344.9) and lowest in T2 (733.4 ± 383.6), showing a P value of 0.021. Furthermore, the activity of splenic LDH level exhibited a similar trend, with T1 (308.5 ± 93.5) significantly elevated over T2 (61 ± 38.81) and T0 (173.7 ± 37.26). However, the ALP activity in the spleen was also increased in fish feed basal diet pellets plus lead nitrate T1 (1250 ± 266.8) and reduced in T0 (623.5 ± 126.5), with a P value of 0.0243. Fish feed diet with QIS supplementation (T2) reduced enzyme fluctuations, particularly in combination with lead exposure (T3), indicating a potential protective effect, with significant differences (P < 0.05) among treatments.

Table 5: Muscle enzyme activity in *C. carpio* exposed to lead and *Q. infectoria* and a combination of both during 60-day intervals

Spleen					
Enzyme activities	**T0 (Control)**	**T1 $Pb(NO_3)_2$**	**T2 (QIS)**	**T3 $Pb(NO_3)_2$ +QIS**	**P value**
ALT (U/L)	67.65±2.65a	116.4±38.65b	65.15±10.85a	76.47±7.143ab	0.0392
AST (U/L)	1714±199.8a	1765±344.9b	733.4±383.6c	1107±382.3abc	0.021
LDH (U/L)	173.7±37.26a	308.5±93.5b	61±38.81c	188±39.11a	0.0404
ALP (U/L)	623.5±126.5a	1250±266.8b	748.5±207.5ab	708.7±294.2ab	0.0243

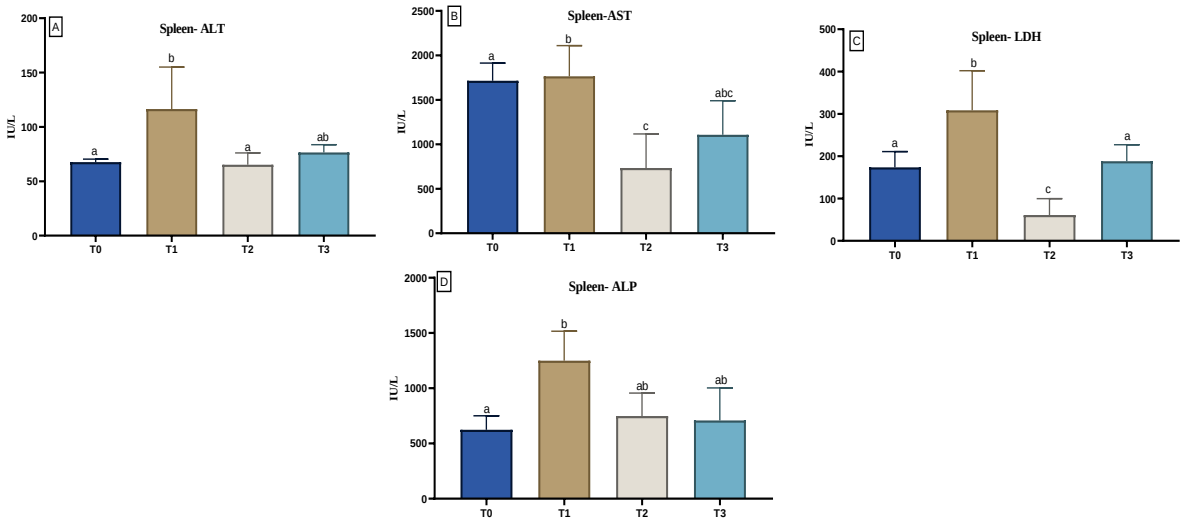

Figure 4: Reveals serum spleen enzyme activity (ALT, AST, LDH, and ALP) in *C. carpio* exposed to lead and *Q. infectoria* and a combination of both during 60-day intervals.

4 Discussion

Control fish maintained expected growth rates, establishing baseline performance standards for comparison [46,47]. $Pb(NO_3)_2$ exposure markedly suppressed growth, consistent with known heavy metal toxicity mechanisms that disrupt metabolic and oxidative processes [48,49]. The observed growth retardation reflects $Pb(NO_3)_2$ interference with critical physiological functions, including nutrient absorption and enzyme activity [50]. Adding QIS to the fish's diet led to impressive outcomes, with the supplemented group growing significantly better than both the untreated fish and those exposed to $Pb(NO_3)_2$. This increase in growth is probably due to the QIS having high nutrient content and the ability to fight oxidative stress, which seems to improve how efficiently the fish use nutrients while protecting their cells [51]. The fish exposed to both QIS and $Pb(NO_3)_2$ showed moderate growth, indicating that QIS supplementation may have the ability to reduce lead's harmful effects. This could be due to *Q. infectoria* ability to reduce oxidative stress caused by this trace element [27]. The results also reveal that QIS could play two important roles in fish farming, enhancing growth while protecting against toxins. Although it shows clear advantages, the fact that it does not completely counteract lead's harmful effects means we still need more research. Future studies should focus on finding the best ways to use it and uncovering how it works at a biological level [52].

On the other hand, Lead-exposed fish (T1) showed pronounced hyperglycemia and elevated urea levels, indicating disrupted glucose metabolism and renal stress, consistent with heavy metal-induced physiological dysfunction [53,54]. The increased liver enzymes (ALT and AST) further confirmed hepatic damage, associated with hepatotoxic effects of lead exposure [55,56]. Conversely, QIS supplementation (T2) significantly improved metabolic profiles, including reduced glucose and urea levels and elevated total protein, suggesting enhanced energy utilization, renal protection, and protein synthesis, likely due to *Q. infectoria* bioactive compounds and balanced amino acid composition [57]. Liver enzyme activities in the QIS and QIS+$Pb(NO_3)_2$ groups remained closer to normal, particularly ALP, indicating partial hepatoprotection through antioxidant mechanisms [58]. Although uric acid levels remained relatively stable across

treatments, the marked improvements in other biochemical markers underline the efficacy of QIS in mitigating heavy metal toxicity. These findings support using *Q. infectoria* seed extract as a functional dietary additive to enhance resilience against environmental contaminants [59].

Lead exposure in carp significantly elevated ALT, AST, LDH, and ALP activities in muscle tissue, indicating cellular damage, membrane destabilization, and metabolic disturbance [40,60]. The marked rise in LDH suggests a metabolic shift towards anaerobiosis, by lead-induced mitochondrial dysfunction and oxidative stress [61,62]. In contrast, QIS supplementation significantly suppressed enzyme activities, even below control levels, reflecting its potent antioxidant and cytoprotective properties [63,64]. A similar trend was observed in the spleen, where lead exposure heightened enzyme activities, implying splenic stress and immune impairment [65,66,67]. Given the spleen's role in immune regulation, such alterations suggest systemic immunosuppression [68,69]. QIS supplementation (T2 and T3) mitigated these effects by modulating inflammatory responses and enhancing antioxidant defenses [70]. Similarly, ALP activity exhibited tissue-specific patterns, decreasing in muscle but varying in spleen, reflecting functional differences across tissues [71,72]. However, QIS's stabilization of enzyme profiles underscores its potential as a functional feed additive to enhance fish health and resilience against environmental pollutants [73,74].

5 Conclusion

Our research article concluded that lead nitrate exposure significantly reduced the growth, disrupted biochemical balance, and induced oxidative stress in *C. carpio*, compromising overall health. However, dietary supplementation with *Q. infectoria* seeds effectively reduces these adverse effects by enhancing growth performance, restoring metabolic stability, and reducing tissue damage through its antioxidant and detoxifying properties. These findings highlight QIS as a promising natural intervention to combat lead toxicity in aquaculture, promoting sustainable fish farming practices. Further research is recommended to optimize QIS inclusion levels and explore its broader applications in heavy metal remediation for aquatic species.

Author Contributions

P. M. S. A. and B.S.A.A.S. contributed to the concept and design of the work. P. M. S. A., B.S.A.A.S., M. O., and O. R. A. were responsible for the acquisition, analysis, and interpretation of data. M.O., B.S.A.A.S., and F.J. drafted the manuscript. All authors reviewed and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Funding

Not applicable.

Availability of data and materials

The corresponding author will provide all data upon reasonable request.

Declarations

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests

References

[1] K. Maheshwari, M. Agrawal, and A. B. Gupta, "Dye Pollution in Water and Wastewater," 2021, 1–25. doi: 10.1007/978-981-16-2892-4_1.

[2] A. K. Qasim, "Novel Synthesis of Gold Doped Silver Phosphate Nanoparticles by Hydrothermal Method for The Enhancement of Visible Light-Responsive Photocatalytic Degradation of Fast Green FCF Dye," *Science Journal of University of Zakho*, **10**(3), 76–80, doi: 10.25271/sjuoz.2022.10.3.916 (Jul. 2022).

[3] S. I. Siddiqui *et al.*, "Investigation of Congo Red Toxicity towards Different Living Organisms: A Review," *Processes*, **11**(3), 807, doi: 10.3390/pr11030807 (Mar. 2023).

[4] M. Berradi *et al.*, "Textile finishing dyes and their impact on aquatic environs," *Heliyon*, **5**(11), e02711, doi: 10.1016/j.heliyon.2019.e02711 (Nov. 2019).
[5] T. S. Vo, M. M. Hossain, T. Lim, J. W. Suk, S. Choi, and K. Kim, "Modification of the interfacial glass fiber surface through graphene oxide-3 chitosan interactions for excellent dye removal as an adsorptive membrane Modification of the interfacial glass fiber surface through graphene oxide-23 chitosan interactions for excellent dye removal as an adsorptive membrane." [Online]. Available: https://ssrn.com/abstract=4156669
[6] X. Yao, P. Huang, and Z. Nie, "Cyclodextrin-based polymer materials: From controlled synthesis to applications," *Prog Polym Sci*, **93**, 1–35, doi: 10.1016/j.progpolymsci.2019.03.004 (Jun. 2019).
[7] H. Agarwal, S. Venkat Kumar, and S. Rajeshkumar, "A review on green synthesis of zinc oxide nanoparticles – An eco-friendly approach," *Resource-Efficient Technologies*, **3**(4), 406–413, doi: 10.1016/j.reffit.2017.03.002 (Dec. 2017).
[8] P. Singh, Y.-J. Kim, D. Zhang, and D.-C. Yang, "Biological Synthesis of Nanoparticles from Plants and Microorganisms," *Trends Biotechnol*, **34**(7), 588–599, doi: 10.1016/j.tibtech.2016.02.006 (Jul. 2016).
[9] J. Singh, T. Dutta, K.-H. Kim, M. Rawat, P. Samddar, and P. Kumar, "'Green' synthesis of metals and their oxide nanoparticles: applications for environmental remediation," *J Nanobiotechnology*, **16**(1), 84, doi: 10.1186/s12951-018-0408-4 (Dec. 2018).
[10] E. N. Yonan, N. M. Sher Mohammed, and A. K. Qasim, "Green synthesis and characterisation of monometallic (Ni) and bimetallic (Ni-Ag) nanoparticles using Cicer Arietinum leaf extract and their applications for adsorption of toluidine blue," *Int J Environ Anal Chem*, 2022, doi: 10.1080/03067319.2022.2118594.
[11] A. Chatterjee, J. Abraham, M. Ajantha, A. Talekar, and N. Revathy, "Biosynthesis, Antimicrobial and Cytotoxic Effects of Titanium Dioxide Nanoparticles Using Vigna unguiculata Seeds," *International Journal of Pharmacognosy and Phytochemical Research*, **9**, 95–99, , doi: 10.25258/ijpapr.v9i1.8047 (Jan. 2017).
[12] I. Khan, K. Saeed, and I. Khan, "Nanoparticles: Properties, applications and toxicities," Nov. 01, 2019, *Elsevier B.V.* doi: 10.1016/j.arabjc.2017.05.011.
[13] L. L. Mustafa and A. K. Qasim, "Facile green synthesis of Mn doped NiO nanoparticles using Vigna unguiculata seed for photocatalytic degradation of toluidine blue," *Int J Environ Anal Chem*, doi: 10.1080/03067319.2025.2487850 (2025).
[14] L. Y. Mohammed, "ANTIOXIDANT ACTIVITY AND PHYSICO-CHEMICAL PROPERTIES OF GREEN SYNTHESIZED ZINC OXIDE NANOPARTICLES USING ERUCA SATIVA LEAF EXTRACT," *Science Journal of University of Zakho*, **11**(4), doi: 10.25271/sjuoz.2023.11.4.1173 (Dec. 2023).
[15] A. A. Goyi, N. M. Sher Mohammad, and K. M. Omer, "Preparation and characterization of potato peel derived hydrochar and its application for removal of Congo red: a comparative study with potato peel powder," *International Journal of Environmental Science and Technology*, **21**(1), 631–642, doi: 10.1007/s13762-023-04965-y (Jan. 2024).
[16] I. Aminu, S. M. Gumel, W. A. Ahmad, and A. A. Idris, "Adsorption Isotherms and Kinetic Studies of Congo-Red Removal from Waste Water Using Activated Carbon Prepared from Jujube Seed," *Am J Analyt Chem*, **11**(01), 47–59, doi: 10.4236/ajac.2020.111004 (2020).
[17] R. Lafi, I. Montasser, and A. Hafiane, "Adsorption of congo red dye from aqueous solutions by prepared activated carbon with oxygen-containing functional groups and its regeneration," *Adsorption Science and Technology*, **37**, (1–2), 160–181, doi: 10.1177/0263617418819227 (Mar. 2019).
[18] A. Pratt, "Environmental applications of magnetic nanoparticles," in *Frontiers of Nanoscience*, **6**, Elsevier Ltd, 2014, 259–307. doi: 10.1016/B978-0-08-098353-0.00007-5.
[19] A. A. Goyi, N. M. Sher Mohammad, and K. M. Omer, "Nitrogen-rich, potato peel-derived hydrochar as an effective dye adsorbent," *Biofuels*, doi: 10.1080/17597269.2024.2336323 (2024).
[20] A. Bavi, M. S. Jafari, M. Heydari, F. Ebrahimi, and A. Sadeghizadeh, "Batch and continuous mode adsorption of methylene blue cationic dye onto synthesized titanium dioxide/polyurethane nanocomposite modified by sodium dodecyl sulfate," *Colloids and Surfaces C: Environmental Aspects*, **1**, 100012, doi: 10.1016/j.colsuc.2023.100012 (Nov. 2023).
[21] R. Islam *et al.*, "Modulating Mn-doped NiO nanoparticles: structural, optical, and electrical property tailoring for enhanced hole transport layers," *Nanoscale Adv*, doi: 10.1039/d4na00708e (Oct. 2024).
[22] V. A. Mane, D. V. Dake, N. D. Raskar, R. B. Sonpir, M. D. Shirsat, and B. N. Dole, "Optimization, kinetic analysis, and photocatalytic degradation of rhodamine B using manganese doped nanoscale nickel oxide nanoparticles," *Ceram Int*, **50**(20), 38871–38883, doi: 10.1016/j.ceramint.2024.07.250 (Oct. 2024).
[23] F. Taghizadeh, "The Study of Structural and Magnetic Properties of NiO Nanoparticles," *Optics and Photonics Journal*, **06**(08), 164–169, doi: 10.4236/opj.2016.68b027(2016).
[24] K. H. J. Buschow, P. G. Van Engen, and R. Jongebreur, "MAGNETO-OPTICAL PROPERTIES OF METALLIC FERROMAGNETIC MATERIALS," 1983.
[25] U. Ahmed, D. Sundholm, and M. P. Johansson, "The effect of hydrogen bonding on the π depletion and the π-π stacking interaction," *Physical Chemistry Chemical Physics*, **26**(43), 27431–27438, doi: 10.1039/d4cp02889a (Oct. 2024).
[26] K. R. Ahmed, D. S. Mohammed, and F. M. Ali, "SYNTHESIS OF A NEW ADSORBENT BASED ON THE MILD DEALUMINATED BINDER FREE GRANULAR Y ZEOLITE," *Science Journal of University of Zakho*, **11**(3), doi: 10.25271/sjuoz.2023.11.3.1115 (Jul. 2023).

[27] P. Palai, S. Muduli, B. Priyadarshini, and T. R. Sahoo, “A facile green synthesis of ZnO nanoparticles and its adsorptive removal of Congo red dye from aqueous solution,” in *Materials Today: Proceedings*, Elsevier Ltd, 2020, 2445–2451. doi: 10.1016/j.matpr.2020.07.387.

[28] I. Ali, Z. A. AL-Othman, and A. Alwarthan, “Molecular uptake of congo red dye from water on iron composite nano particles,” *J Mol Liq*, **224**, 171–176, doi: 10.1016/j.molliq.2016.09.108 (Dec. 2016).

[29] H. Zhu, W. Wang, Y. Huo, X. He, H. Zhao, and H. Wang, “Molecular Simulation Study on Adsorption and Diffusion Behaviors of CO2/N2in Lignite,” *ACS Omega*, **5**(45), 29416–29426, doi: 10.1021/acsomega.0c04352 (Nov. 2020).

[30] X. Yang, X. Cao, C. Chen, L. Liao, S. Yuan, and S. Huang, “Green Synthesis of Zinc Oxide Nanoparticles Using Aqueous Extracts of Hibiscus cannabinus L.: Wastewater Purification and Antibacterial Activity,” *Separations*, **10**(9), doi: 10.3390/separations10090466 (Sep. 2023).