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Mannan Oligosaccharides Biotherapeutic Effect Against Genotoxicity and Growth Suppression Caused by Lead inTilapia

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ABSTRACT

Exposure to lead (Pb) through aquaculture poses significant health risks to aquatic species and the environment. To help safeguard aquaculture health and sustainability, prebiotics have emerged as a promising strategy. This study investigated the protective effect of mannan oligosaccharides (MOS) on Pb-induced growth inhibition and DNA damage in the juvenile Nile tilapia (*Oreochromis niloticus*). Fish were divided into six treatment groups: a contaminant-free control, 10 ppm Pb exposure, MOS supplementation at 0.3% and 0.6% of diet, and combined Pb exposure with MOS supplementation (0.3% and 0.6%). DNA damage was assessed using the comet assay, and growth performance was monitored throughout the feeding trial. Tilapia exposed to Pb alone showed significant DNA damage, evidenced by increased comet tail length and moment, and reduced growth rates compared to other groups. In contrast, fish fed MOS-supplemented diets exhibited improved feed intake, higher weight gain, and reduced DNA damage, even when exposed to Pb. These findings indicate that MOS supplementation may mitigate the adverse effects of lead exposure by enhancing growth and reducing genotoxic stress.

INTRODUCTION

In Egypt, the Nile tilapia (*Oreochromis niloticus*) is a vital species for commercial fishing. It's a common freshwater fish that can survive in highly contaminated environments **(Carvalho *et al.*, 2012; Al-Asgah *et al*., 2015*;* Hashem, 2022; Heiba *et al.*, 2025a).**

According to **Mehrim and Refaey (2023)**, pond-based fish is an essential source of fish farming in the Nile Delta's lakes, as it accounts for 85% of Egypt's total fish production. Egypt produces over 73.8% of the fish raised in Africa and ranks tenth in the world in terms of total cultured fish output, with an annual production exceeding millions of tonnes **(Al-Wakeel *et al*., 2019; FAO, 2022)**. Globally, tilapia is the most extensively farmed fish group, with total production reaching 6.3 million metric tonnes in 2021, valued at over USD 12 billion **(FAO, 2023)**.

On the other hand, one of the most hazardous threats for tilapia in Egypt is water contamination with toxic compounds, particularly heavy metals, produced from industrial, urban, and agricultural activities **(Abdel-Mohsien & Mahmoud, 2015; Heiba *et al.*, 2021, 2025b)**. Lead (Pb), as a heavy metal, is one of the 129 priority pollutants designated by the Environmental Protection Agency and acts as a cumulative toxin **(Kumar *et al*., 2007; Rajeshkumar *et al*., 2017)**. Exposure to lead has a variety of harmful consequences on an animal's physiological, behavioral, and biochemical processes **(Hsu & Guo, 2002)**. Furthermore, it can severely damage various systems, including the reproductive, cardiovascular, hematopoietic, and central nervous systems and damage may extend to organs such as liver and kidney **(Flora *et al*., 2012; Subotić *et al*., 2013; *Bruce et al*., 2024; Adriana *et al*., 2025)**. Among the naturally occurring forms of lead is lead acetate which is considered one of the most hazardous **(Ara *et al*., 2015)**.

Lead accumulates in the liver, where it forms lead–bile complexes to facilitate its elimination. Like other heavy metals, lead damages the liver through oxidative stress caused by reactive oxygen species (ROS), particularly when the antioxidant cellular defense system is unable to prevent excessive ROS generation **(Kim & Kang, 2015, 2017a)**. According to *in vitro*, *in vivo*, and human investigations **(García-Lestón *et al*., 2010)**, lead is a strong genotoxic substance that can result in chromosomal abnormalities, sister chromatid exchange, DNA damage, and the production of micronuclei. Hydrogen peroxide, as a ROS member, turns into a hydroxyl radical, which results in oxidative stress, protein damage, lipid peroxidation, and DNA/RNA damage **(Kim & Kang, 2017b; Dey *et al*., 2025)**.

To improve tilapia productivity and sustainability, it is essential to provide diets that are growth-promoting, immunostimulatory, and rich in antioxidants **(Eissa *et al*., 2024)**. In aquaculture, prebiotics have become safe and environmentally beneficial functional dietary additives **(El-Nobi *et al*., 2021; Wang *et al*., 2022).** These prebiotics are indigestible dietary components derived from carbohydrates and yeast cell walls. They primarily include oligosaccharides such as fructo-oligosaccharide (FOS), galacto-oligosaccharide (GOS), xylo-oligosaccharide (XOS**)** and mannan-oligosaccharide (MOS) **(Hendam *et al*., 2023)**. As stated by **Harikrishnan *et al*. (2023)**, MOS is widely used in aquaculture as an effective prebiotic. Dietary MOS has been demonstrated to improve growth rates **(Selim & Reda, 2015; Azevedo *et al*., 2016)**, enhance the activity of the antioxidant cellular defense system in the Nile tilapia **(El-Nobi *et al*., 2021; Mohanty, 2024)**, control glucose and lipid metabolism, and change gut microbiota in fish fed a high-carbohydrate diet **(Wang *et al*., 2022)**. It has been demonstrated that these substances stimulate the growth of beneficial gut bacteria **(**probiotics) **(Nawaz *et al*., 2018)**. Probiotics can lessen the negative effects of lead toxicity in fish **(Kirillova *et al*., 2017)**. Thus, this study aims to investigate the protective effect of MOS biotherapeutic as a prebiotic in the Nile tilabia (*Oreochromis niloticus*) against the genotoxicity and growth suppression of lead.

MATERIALS AND METHODS

The experiment started in July 2023 at the Genetic Engineering and Biotechnology Research Institute, University of Sadat City. The experimental juvenile Nile tilapia were obtained from a government hatchery. Eighteen rectangular glass tanks (27 × 22 × 86 cm) each with a water volume of 52L, were used. Each tank housed 20 juvenile tilapia with an average initial weight of 18±0.64g. Fish were acclimatized for 14 days in water maintained at 28°C with a 12h light/12h dark cycle. They were fed twice daily to apparent satiation. Water quality (pH, ammonia, nitrites, and dissolved oxygen) was monitored regularly.

Experimental treatments

After acclimatization, the fish were divided into six groups, each replicated in three tanks, and the total experimental duration was 60 days. Water was replaced every three days to maintain water quality and treatment concentration. One group was used as a control without any treatment and fed on a commercial diet, and two groups were used for the treatment with two concentrations of MOS (0.3 and 0.6% of the diet). The other three groups were used for lead treatments. One of these fish groups was reared in water with 10 ppm of lead and fed on a control diet as reported by **El-Fahla *et al*. (2022b)**, and the last two groups were fed on a diet supplemented with the two concentrations of MOS (0.3 and 0.6% of the diet).

I. Growth evaluation

The growth performance was evaluated by the determination of the specific growth rate (SGR). Fish were weighed at the start of the experiment, at day 30 and at day 60 at the end of experiment.

II. Genotoxicity assessment by comet assay

The liver cells were subjected to the comet assay (single cell gel electrophoresis) to determine the quantity of DNA damage of Pb-exposed fish liver tissues and to identify the protective effect of MOS. The alkaline comet assay was applied according to **Singh *et al.* (1988)**. Liver cell suspension (10µL) was mixed with 90µL of 1% low melting point agarose solution in phosphate buffer saline (PBS, pH 7.2) at 37°C and pipetted onto fully frosted microscope slides, which contained a layer of 1% normal agarose. The liver cells were then lysed by immersing the slides in a lysing buffer solution pH:10 (2.5 M NaCl, 100 mM Na_2-EDTA, 10 mM Tris base with freshly added 1% Triton X-100 and 10µg/ mL Proteinase K) at 37°C overnight to remove the proteins. The slides were horizontally incubated in a freshly prepared cold alkaline buffer (4°C, 200 mM Na_2-EDTA, 10 N NaOH, pH > 13.0) for 20 minutes to relax DNA and have expression of alkali-labile sites and single strand breaks. Alkaline buffer

electrophoresis was performed for approximately 10 minutes (~280 V, 25 A) using a compact power supply. The slides were neutralized after electrophoresis by washing three times in a 0.4 M Tris buffer (pH 7.5), air-dried and stained with the fluorescent dye Ethidium Bromide (20µg/ mL). Washed stained slides were kept in the dark at 4°C. A total of 500 randomly stained cells (liver nuclei) were observed, captured and analyzed using a LEICA DM3000 fluorescence microscope (magnification 400×). The microscope was connected through a LEICA EC3 camera to a computer-based image analysis system (Comet Assay IV Software, Perspective Instruments). The main parameters of comet imaging were tail length, head intensity and olive tail moment.

Statistical Analysis

Statistical analyses were conducted on the data using one-way analysis of variance (ANOVA) followed by the Duncan's test for multiple comparisons. A significance level of $P< 0.05$ was statistically significant. All statistical analyses were performed in SPSS (v. 25.0).

RESULTS

The treatment effects on the specific growth rate (SGR)

As illustrated in Fig. (1), the results indicated that, on the 30th day post-treatment, differences in SGR were observed among the experimental groups. Fish in the MOS (0.3%) and MOS (0.6%) groups exhibited the highest SGR values of 2.28 and 2.25% compared to the control group (1.85%). However, fish in the lead group showed a reduction in SGR (1.15%) compared to the control. Lead with different concentrations of MOS (0.3% and 0.6%) groups showed a moderate increase (1.76% and 1.85%, respectively). On the 60th day, a similar trend was observed with the MOS 0.3 and 0.6% groups that displayed the highest SGR values (2.22 and 2.20%) compared to the control group (2.10%). An increase in SGR (1.42%) was observed in the lead group compared to its value at day 30, albeit lower than that of the control and MOS groups. Meanwhile, the lead + MOS (0.3% and 0.6%) groups demonstrated further improvements (1.91% and 1.94, respectively).

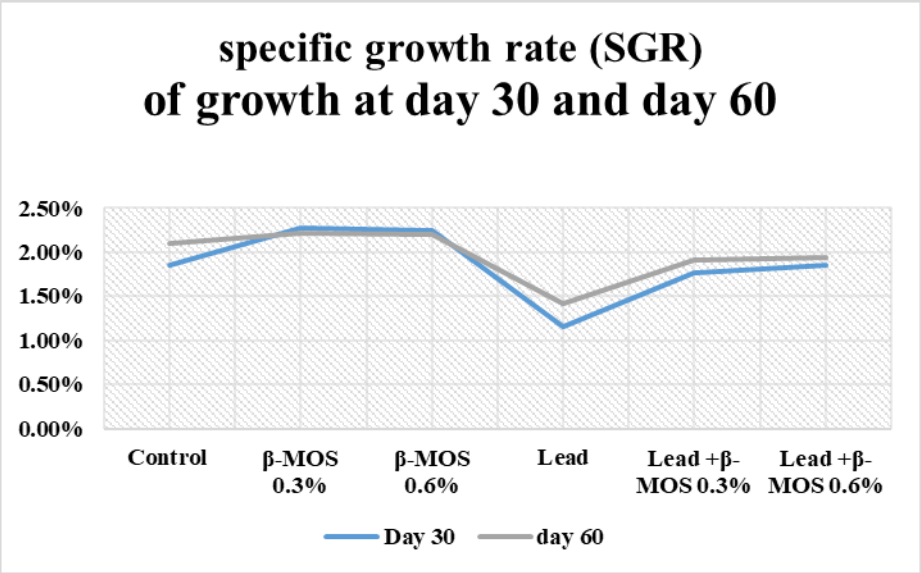

Fig. 1. Specific growth rate (SGR) of the Nile tilapia after exposure to lead (10ppm) and treated with MOS (0.3% and 0.6%) at days 30 and 60

The treatment effect on the total survival rate (TSR)

As illustrated in Fig. (2), in the different groups, the survival rates were high **on the 30th day** after treatment, with the control group exhibiting 100%. The MOS (0.3%) and (0.6%) groups showed a minor decline of 99.3%. A larger decline in the survival rate was recorded in the lead group (98%). On the other hand, in the two groups of lead + MOS treatments (0.3% and 0.6%), the survival rate was 98.7% for both. In every group, survival rates decreased somewhat by the **60th day**. In the control group, the survival rate was 99.3%. In addition, in the two concentrations of the MOS (0.3% and 0.6%), the survival rate was recorded at 98.7% and 98%, respectively. In the lead group, the survival rate was 95.3%. However, in the lead + MOS (0.3% and 0.6%) groups, the survival rates of 97.3% and 98% were observed.

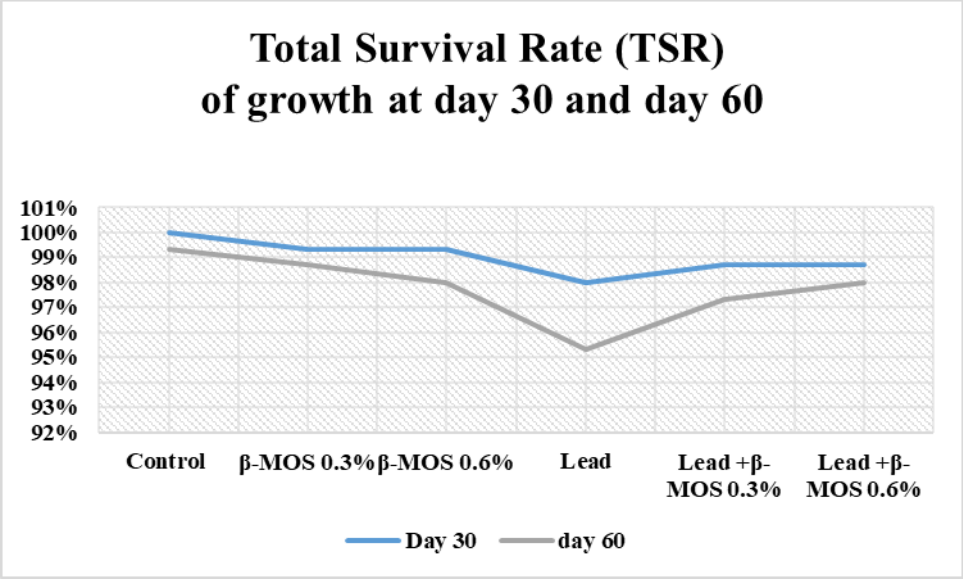

Fig.(2): Total Survival Rate (TSR) of Nile tilapia after exposure to the lead (10 Ppm) and treated with MOS (0.3% and 0.6%) on days 30 and 60.

The treatment effect on the tail length of comet

The comet assay grading (grades 0-4) is shown in Fig. (3). On the **30th day** post-treatment, significant increases (P<0.05) in the tail length were observed in fish exposed to lead and lead + MOS (0.3% and 0.6%) groups (27.90±1.23, 15.40±0.80, and 14.30±0.53, respectively) when compared to the control and MOS (0.3% and 0.6%) groups (6.70±0.26, 7.20±0.35, and 6.90±0.45 , respectively) as observed in Fig. (4).

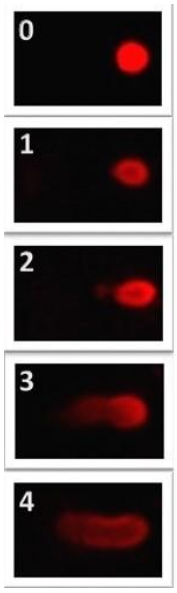

Fig. 3. Comet assay grading (grades 0-4)

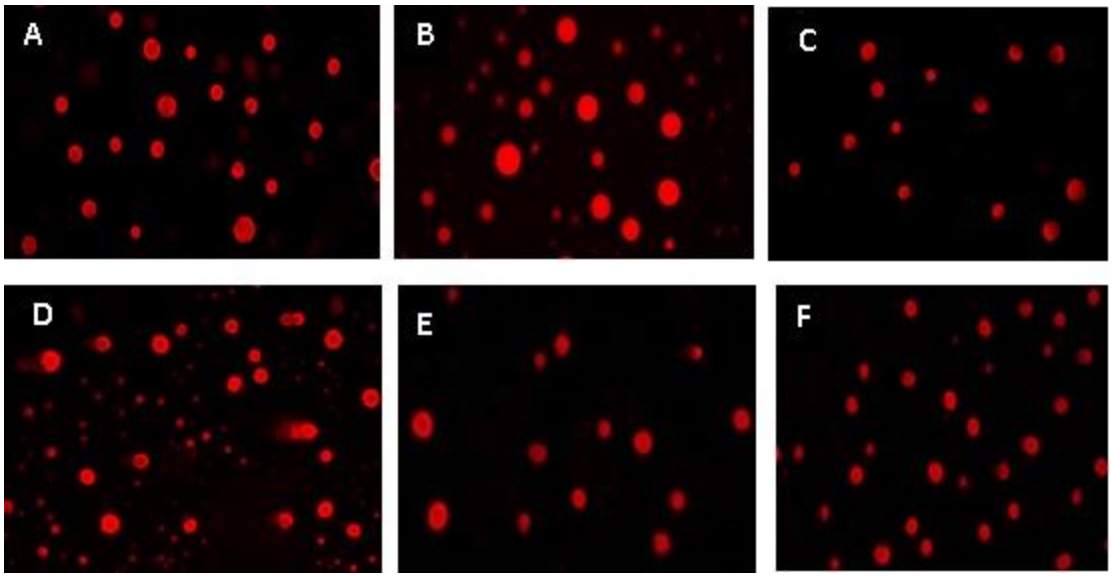

Fig. 4. Fluorescence photomicrograph (× 400) of liver cells of the Nile tilapia at day 30 showing the comet assay and stained with ethidium bromide. A) Negative control group; (B) 0.3% MOS group; (C) 0.6% MOS group; (D) Lead group; (E) Lead + 0.3% MOS group; and (F) Lead + 0.6% MOS group

On the **60th day**, a similar trend was observed in the tail length of comet, with a significant increase (P<0.05) in tail length in the lead-treated groups (33.90±0.73, 18.70±0.78, and 17.90±1.02) compared to the control, MOS (0.3% and 0.6%) groups that were 6.90±0.27, 7.10±0.34, and 7.00±0.44, respectively (Fig. 5).

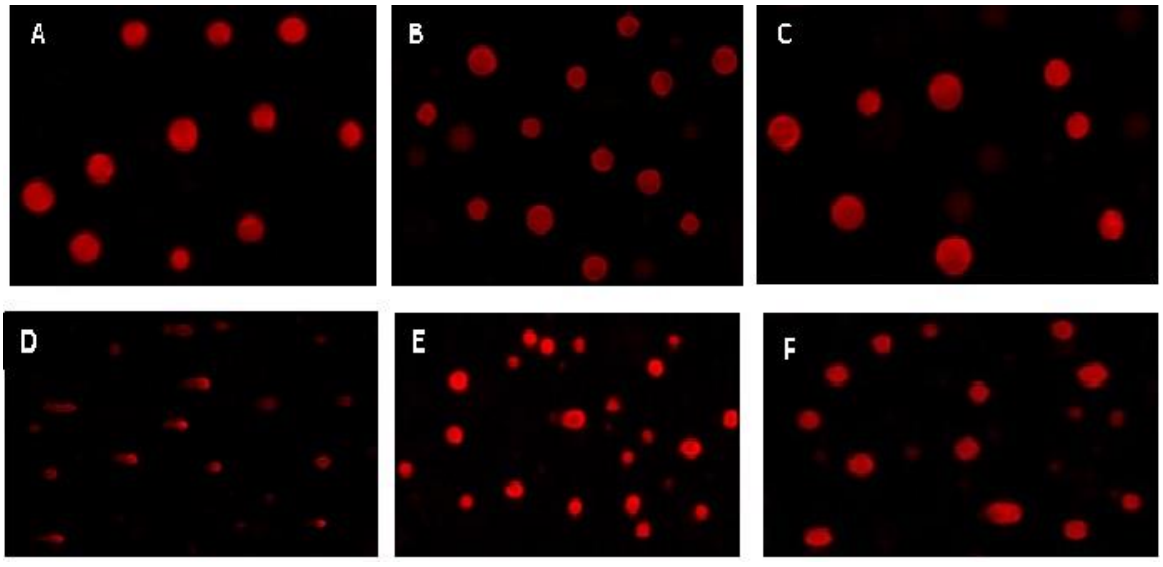

Fig. 5. Fluorescence photomicrograph (× 400) of liver cells of the Nile tilapia at day 60 showing the comet assay and stained with ethidium bromide. A) Negative control group; (B) 0.3% MOS group; (C) 0.6% MOS group; (D) Lead group; (E) Lead + 0.3% MOS group; and (F) Lead + 0.6% MOS group

The duration effect on tail length showed that the tail length in the lead, lead + MOS (0.3%), and lead + MOS (0.6%) groups significantly increased ($P< 0.05$) at day 60 compared to day 30, as presented in Fig. (6).

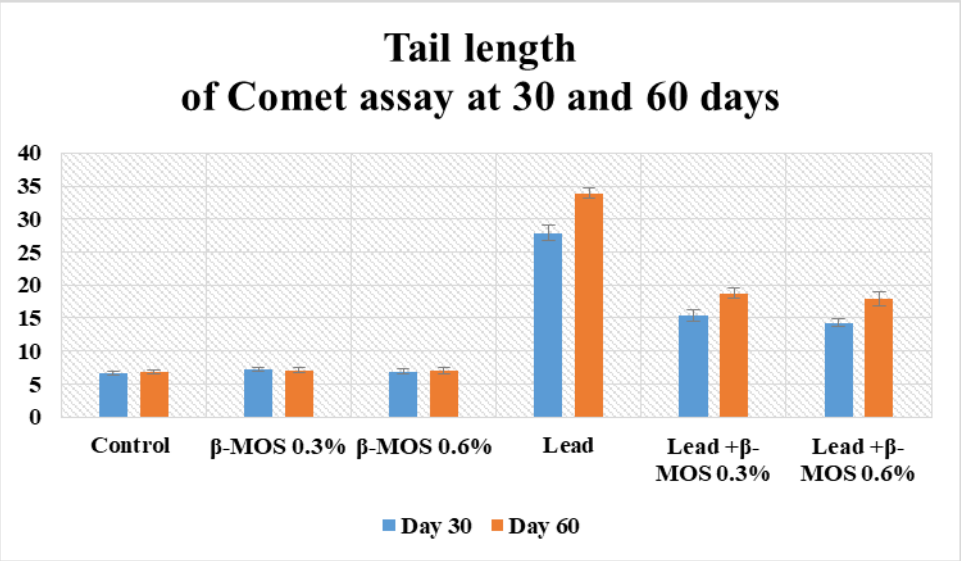

Fig. 6. Tail length in the comet assay (mean ± SE) on liver cells of the Nile tilapia after exposure to lead (10 Ppm) and treated with MOS (0.3% and 0.6%) at days 30 and 60

The treatment effect on the head intensity of the comet

On the **30th day** post-treatment, the head intensity shows a significant ($P<0.05$) decrease in the treated groups compared to the control (99.78±0.14). In the lead group and lead + MOS (0.3% and 0.6%) groups, the head intensity was 92.03±2.00, 94.40±1.87, and 96.78±2.15, respectively. In the MOS (0.3% and 0.6%) groups, the head intensity was 99.92±0.07, and 99.92±0.03, respectively. This significant decrease ($P< 0.05$) was still evident on the 60th day, with the fish treated with lead showing reduced head intensity (89.62±1.29, 93.35±2.01, and 94.92±2.39, respectively) compared to the control, MOS (0.3%), and MOS (0.6%) groups (99.65±0.17, 99.85±0.09, and 99.90±0.03, respectively).

The Impact of the treatment duration on the head intensity illustrates that the head intensity in fish groups treated with lead significantly decreased ($P< 0.05$) at day 60 compared to day 30, as shown in Fig. (7).

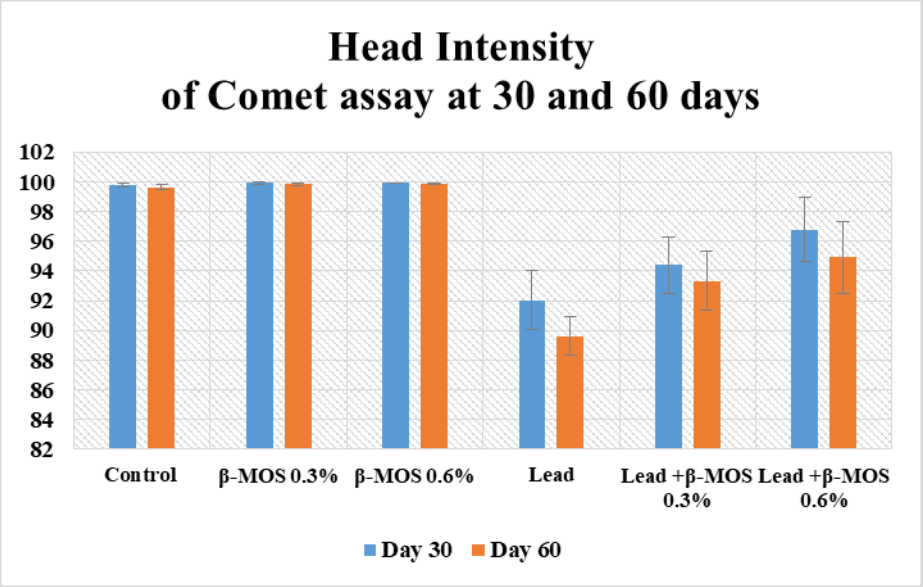

Fig. 7. The head intensity in the comet assay (mean ± SE) on liver cells of the Nile tilapia after exposure to lead (10 Ppm) and treated with MOS (0.3% and 0.6%) at days 30 and 60.

Treatment effect of the olive tail moment of the comet

As illustrated in Fig. (5), On the **30th day** post-treatment, the olive tail moment revealed a significant increase (P<0.05) in fish exposed to the lead, lead + MOS (0.3% and 0.6%) groups (2.24±0.51, 0.92±0.31, and 0.51±0.36, respectively) compared to the control and MOS (0.3% and 0.6%) groups that were 0.01±0.01, 0.00±0.00, and 0.00±0.00). On the **60th day**, significant differences (P< 0.05) were again observed in the lead-treated groups (3.48±0.41, 1.22±0.39, and 0.92±0.43, respectively) compared to the control, MOS (0.3% and 0.6%) groups (0.02±0.01, 0.01±0.00, and 0.00±0.00, respectively).

The time-dependent effect on the olive tail shows that the olive tail moment significantly increased (P<0.05) from day 30 to day 60 in the lead group, with a similar trend detected in the lead + MOS (0.3% and 0.6%) groups, as presented in Fig. (8).

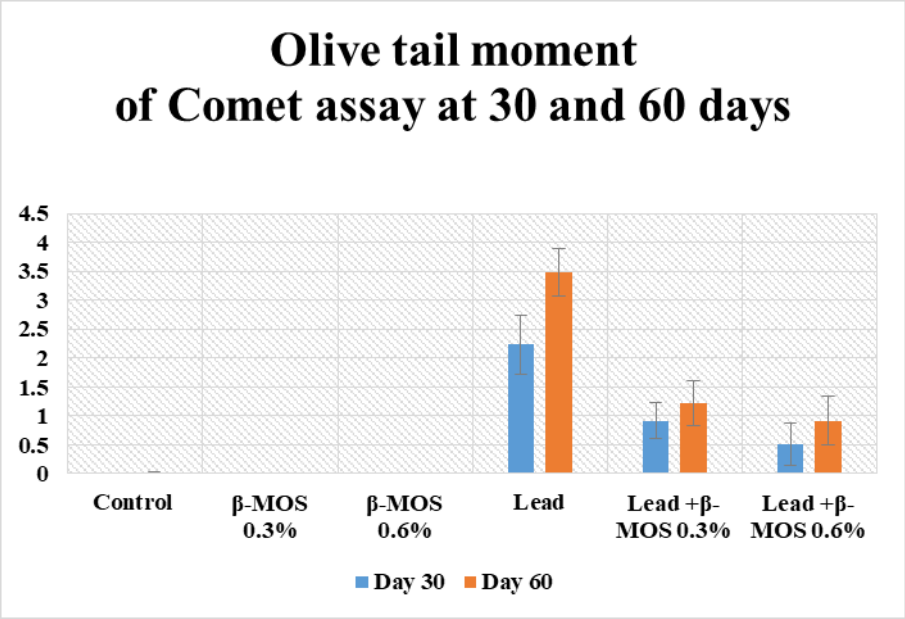

Fig. 8. Olive tail moment in the comet assay (mean ± SE) on the liver cells of the Nile tilapia after exposure to the lead (10 Ppm) and treated with MOS (0.3% and 0.6%) at days 30 and 60

DISCUSSION

Lead is one of the major environmental contaminants affecting aquatic ecosystems worldwide. Through bioaccumulation in the food chain, Pb poses a threat to human health and causes harmful damage to fish **(Zhai *et al.*, 2017; *Bruce et al.*, 2024; Adriana *et al.*, 2025)**.

Recently, there was a growing push to reduce the harmful impacts of heavy metals on farmed fish by utilizing ecologic feed additives rather than antibiotics **(Giri *et al.* 2021)**. Although there is currently no proven therapy for lead toxicity in aquaculture, the application of MOS, as a prebiotic, has received considerable attention due to its beneficial effects on health **(Dimitroglou *et al.*, 2010; Ringø *et al.*, 2010; Asbury & Saville, 2025)**.

The current study evaluated the genotoxic effects of lead (Pb) exposure in the Nile tilapia (*Oreochromis niloticus*) and its impact on growth, as well as the protective effect of different concentrations of MOS (0.3% and 0.6% of diet) at day 30 and day 60 post-treatment.

Exposure of the Nile tilapia to 10ppm Pb resulted in a reduction in growth performance, indicated by a decline in SGR, beside significant elevations in comet assay parameters (tail length, head intensity, olive tail moment), reflecting pronounced DNA breaks. The severity of DNA damage peaked at day 30 and day 60 post-treatment. MOS supplementation improved growth and reduced genotoxicity in both MOS doses, 0.3% and 0.6%.

These findings support the results of previous studies that reported growth inhibition in fish exposed to Pb that causes oxidative stress and leads to decreased feed intake, energy redirection toward tissue repair, reduced feed efficiency and impaired overall growth performance **(Ding *et al*., 2019; Giri *et al*., 2021; Aziz *et al*., 2025)**. Furthermore, lead-induced genotoxicity has been reported in various studies, evidenced either by the comet assay **(Chatha *et al*., 2023; Sharma *et al*., 2025)** or by micronucleus test **(Maryam *et al*., 2023; Dey *et al*., 2024)**. Pb exposure induces excessive production of ROS, leading to oxidative stress **(Mondal *et al*., 2019; Shaw *et al*., 2019; Dey *et al*., 2025)**. The generation of ROS is a major pathway through which lead causes genotoxicity. Concurrently, Pb weakens natural antioxidant defenses, shifting the cellular redox balance toward oxidative stress **(Matović *et al*., 2015)**.

On the other hand, several studies have demonstrated that the dietary supplementation with MOS enhances growth performance in the Nile tilapia (**Wang, T. *et al*., 2022; Al-Ghamdi *et al*., 2023; moustafa *et al.*, 2024; Zhang *et al.*, 2025**). In addition, MOS supplementation has been reported to reduce genotoxicity **(Hafner *et al.*, 2019)**, who demonstrated that MOS attenuated DNA damage as assessed by the comet assay and supported the results of the present study. **Lu *et al*. (2021)** and **El-Fahla *et al*. (2022a)** found that MOS greatly decreased oxidative stress in Pb-exposed fish. Overall, MOS can enhance gut health by promoting better intestinal development and increasing the nutrient absorption surface area **(Zhang *et al.*, 2025)**. Moreover, MOS possesses strong antioxidant properties, as it scavenges ROS and restores cellular redox equilibrium **(Ren *et al.*, 2020)**.

Other previous research has shown that supplementation of the Nile tilapia diets with MOS and glucan did not improve growth performance or feed utilization **(Whittington *et al.*, 2005; Sado *et al.*, 2008; Shelby *et al.*, 2009)**. The delay in growth was attributed to gastric inflammation in the common carp (*Cyprinus carpio*) induced by glucan **(Lin *et al.*, 2011)**.

The variability in the results of different studies, including the present study, can be explained by the complex carbohydrate structures present in the yeast cell wall, differences in yeast strains, fermentation conditions, MOS concentration, administration period, fish developmental stage, rearing conditions and feed formulation, all of which can alter their functional properties **(Newman, 2007; Peterson *et al*., 2012; Torrecillas *et al*., 2014)**.

CONCLUSION

Lead exposure harms the growth and genetic stability of the Nile tilapia. Supplementation with MOS improved growth performance, especially specific growth rate, and reduced DNA damage. These results suggest that MOS is a promising and environmentally friendly feed additive to protect fish health in lead-contaminated

environments. Further studies should explore its molecular mechanisms and long-term impacts under real aquaculture conditions for promoting fish health and productivity in polluted aquatic ecosystems.

REFERENCES

Abdel-Mohsien, H. S. and Mahmoud, M. A. (2015). Accumulation of some heavy metals in *Oreochromis niloticus* from the Nile in Egypt: potential hazards to fish and consumers. Journal of Environmental Protection, 6(09), 1003.

Adriana, G.; Rojas-Lemus; M., López-Valdez; N., Bizarro-Nevares, P.; Morales-R. and Casarrubias-Tabarez B. (2025). Lead systemic toxicity: A persistent problem for health, Toxicology, 515, 154163.

Al-Asgah N.A.; Abdel-Warith A.-W.A.; Younis E.-S.M. and Allam H.Y. (2015) Haematological and biochemical parameters tissue accumulations cadmium in and of *Oreochromis niloticus* exposed to various concentrations of chloride. Saudi cadmium Journal of Biological Sciences, 22, 543-50.

Al-Ghamdi, O. A.; Saïdi, S. A.; Farhat-Khemakhem, A.; Ghorbel, S.; Chouayekh, H.; Cerruti, P. and Azaza, M. S. (2023). Exogenous Propeller Phytase and Prebiotic Mannan Oligosaccharide (MOS) Supplementation of Formulated Diets Applied to Juvenile Nile Tilapia, *Oreochromis niloticus*: Impact on Growth Performance and Nutrient Digestibility. Fishes, 8(12), 574

Al-Wakeel, A.H.; Zahran, E.; Hafez, E.E.; Hamed, M. and Zaki V.H. (2019). Impacts of mannan oligosaccharides (MOS) on growth performance and gastrointestinal health of Nile tilapia (*Oreochromis niloticus*). Manufia . Vet. Med. journal 20(3), 1–7.

Ara, A. and Usmani, J. A. (2015). Lead toxicity: A review. Interdisciplinary toxicology, 8(2), 55.

Asbury, R. and Saville, B. A. (2025) Manno-oligosaccharides as a promising antimicrobial strategy: pathogen inhibition and synergistic effects with antibiotics. Front. Microbiol. 16:1529081.

Azevedo, R. V. D.; Fosse Filho, J. C.; Pereira, S. L.; Cardoso, L. D.; Andrade, D. R. D. and Vidal, M. V. (2016). Dietary mannan oligosaccharide and Bacillus subtilis in diets for Nile tilapia (*Oreochromis niloticus*). Acta Scientiarum. Animal Sciences, 38(4), 347-353.

Aziz, Q.; Nasir, M. H. M.; Jusoh, A. R.; Ahmad, A. H., Othman, Z.; Ahmi, A. and Zakaria, R. (2025) ARTICLE INFO ABSTRACT.

Bruce, L.; Ana, N. and Bellinger D. C. (2024). Lead Poisoning. *New England Journal of Medicine*, 391(17), 1621–1631.

Carvalho C.d.S.; Bernusso V.A.; Araújo H.S.S.d.; Espíndola E.L.G. and Fernandes M.N. (2012). Biomarker responses as indication of contaminant effects in *Oreochromis niloticus*. Chemosphere, 89, 60-9.

Chatha, A. M. M.; Naz, S.; Iqbal, S. S.; Kiran, A.; Lateef, M.; Zahra, U. and Javaid, A. (2023). Detection of DNA damage in fish using comet assay. Current Trends in OMICS, 4(1), 01-16.

Dey, K. K.; Mondal, P. and Chattopadhyay, A. (2024). Environmentally relevant lead alters nuclear integrity in erythrocytes and generates oxidative stress in liver of Anabas testudineus: involvement of Nrf2-Keap1 regulation and expression of biomarker genes. Journal of Applied Toxicology, 44(2), 260-271.

Dey, K. K.; Kamila, S.; Das, T. and Chattopadhyay, A. (2025). Chronic exposure to lead causes neurotoxicity by generating oxidative stress and inducing DNA damages in zebrafish brain: involvement of Nrf2-Keap1 regulation and DNA repair pathways. Biological Trace Element Research, 1-13.

Dimitroglou, A. D.L.; Merrifield, P.; Spring, J.; Sweetman, R. Moate and S.J. Davies.(2010). Effects of mannan oligosaccharide (MOS) supplementation on growth performance, feed utilization, intestinal histology and gut microbiota of gilthead sea bream (Sparus aurata). Aquaculture, 300: 182-188.

Ding Z.; Kong Y.; Shao X.; Zhang Y.; Ren C.; Zhao X.; Yu W.; Jiang T. and Ye J. (2019) Growth, antioxidant capacity, intestinal morphology, and metabolomic responses of juvenile Oriental river prawn (Macrobrachium nipponense) to chronic lead exposure. Chemosphere, 217, 289-97

Eissa, E. S. H., Bazina, W. K.; Abd El-Aziz, Y. M.; Abd Elghany, N. A.; Tawfik, W. A.; Mossa, M. I. and Khalil, H. S. (2024). Nano-selenium impacts on growth performance, digestive enzymes, antioxidant, immune resistance and histopathological scores of Nile tilapia, *Oreochromis niloticus* against *Aspergillus flavus* infection. Aquaculture International, 32(2), 1587-1611.

El-Fahla, N. A.; Khalil, K. A.; Dessouki, A. A.; Abdelrazek, H. M.; Mohallal, M. E. and El-Hak, H. N. G. (2022a). Dietary Beta-MOS® ameliorated lead induced reproductive toxicity and stress in Nile tilapia. Aquaculture, 548, 737711.

El-Fahla, N. A.; Mohallal, M. E.; Gad El-Hak, H. N.; Dessouki, A. A. and Abdelrazek, H. (2022b). Effect of lead toxicity on the kidney of Nile tilapia:

amelioration by Beta-MOS®. Egyptian Academic Journal of Biological Sciences, D. Histology and Histochemistry, 14(2), 31-45.

El-Nobi, G.; Hassanin, M.; Khalil, A. A.; Mohammed, A. Y.; Amer, S. A.; Montaser, M. M. and El-Sharnouby, M. E. (2021). Synbiotic effects of Saccharomyces cerevisiae, mannan oligosaccharides, and glucan on innate immunity, antioxidant status, and disease resistance of Nile tilapia, *Oreochromis niloticus*. Antibiotics, 10(5), 567.

FAO (Food and Agriculture Organization). (2022). The state of world fisheries and aquaculture 2022. Towards blue transformation. Rome, Italy: FAO.

FAO (Food and Agriculture Organization). (2023). Global Aquaculture Production Quantity (1950 - 2021). FAO, Rome, Italy.

Flora, G.; Gupta, D. and Tiwari, A. (2012). Toxicity of lead: a review with recent updates. Interdisciplinary toxicology, 5(2), 47.

García-Lestón, J.; Méndez, J.; Pásaro, E. and Laffon, B. (2010). Genotoxic effects of lead: an updated review. Environment international, 36(6), 623-636.

Giri S.S.; Kim M.J.; Kim S.G.; Kim S.W.; Kang J.W.; Kwon J.; Lee S.B.; Jung W.J.; Sukumaran V. and Park S.C. (2021) Role of dietary curcumin against waterborne lead toxicity in common carp Cyprinus carpio. Ecotoxicology and Environmental Safety ,219, 112318.

Hafner, D.; Tuboly, T.; Mézes, M.; Bloch-Bodnár, Z.; Balogh, K.; Vántus, V. and Kovács, M. (2019). Effect of feeding Bacillus cereus var. toyoi and/or mannan oligosaccharide (MOS) on blood clinical chemistry, oxidative stress, immune response and genotoxicity in T-2 toxin exposed rabbits. Italian Journal of Animal Science, 18(1), 1239-1251.

Harikrishnan, R.; Devi, G.; Balamurugan, P.; Abdel-Warith, A. W. A.; Younis, E. M.; Van Doan, H. and El-Haroun, E. (2023). Immunostimulatory effect of mannan-oligosaccharides supplementation diet in milkfish (*Chanos chanos*). Fish and Shellfish Immunology, 133, 108568.

Hashem, M. H. (2022). Early sexing of *Tilapia nilotica* (*Oreochromis niloticus*) by using short sequence repeats (SSRs) molecular markers. Damanhour Journal of Veterinary Sciences, 8, 22–25.

Heiba, A. A.; Amal, M. Y.; Ebrahimet, A. S.; Reham, M. A. and Medhat, H. H. (2021). Biochemical, histopathological, and genetic impacts of River Nile

pollutants on the Nile tilapia (*Oreochromis niloticus*). Egyptian Journal of Aquatic Biology and Fisheries, 25(3), 883–899.

Heiba, A.; Hashem, M.; El-Kasheif, M.; Sabra, E. and Gad, A. (2025a). Possible Beneficial Molecular Effects of Nano-Chitosan against Manganese Genotoxicity in the Nile Tilapia (*Oreochromis niloticus*) using SCoT Molecular Markers. 29. 1709-1725.

Heiba, A.; Tayel, S.; Kasheif, M.; Sabra, E. and Hashem, M. (2025b). Histopathological assessment of heavy metal toxicity in the gonads of Nile tilapia (*Oreochromis niloticus*) from the Rosetta branch, Nile River, Egypt. African Journal of Biological Sciences 21(1):47-57.

Hendam, B. M.; Munir, M. B.; Eissa, M. E.; El-Haroun, E.; van Doan, H.; Chung, T. H. and Eissa, E. S. H. (2023). Effects of water additive probiotic, Pediococcus acidilactici on growth performance, feed utilization, hematology, gene expression and disease resistance against *Aspergillus flavus* of Nile tilapia (*Oreochromis niloticus*). Animal Feed Science and technology, 303, 115696.

Hsu, P. C. and Guo, Y. L. (2002). Antioxidant nutrients and lead toxicity. Toxicology, 180(1), 33-44.

Kim, J.H. and Kang, J.C. (2015). Oxidative stress, neurotoxicity, and non-specific immune responses in juvenile red sea bream, Pagrus major, exposed to different waterborne selenium concentrations. Chemosphere 135, 46–52.

Kim, J.H. and Kang, J.C. (2017a). Effects of dietary chromium exposure to rockfish, Sebastes schlegelii are ameliorated by ascorbic acid. Ecotoxicol. Environ. Saf., 139, 109–115.

Kim, J.H. and Kang, J.C. (2017b). Effects of sub-chronic exposure to lead (Pb) and ascorbic acid in juvenile rockfish: antioxidant responses, MT gene expression, and neuro transmitters. Chemosphere, 171, 520–527.

Kirillova, A. V.; Danilushkina, A. A.; Irisov, D. S.; Bruslik, N. L.; Fakhrullin, R. F.; Zakharov, Y. A. and Yarullina, D. R. (2017). Assessment of resistance and bioremediation ability of *Lactobacillus* strains to lead and cadmium. International journal of microbiology, (1), 9869145.

Kumar, P.; Prasad, Y.; Patra, A. K. and Swarup, D. (2007). Levels of cadmium and lead in tissues of freshwater fish (*Clarias batrachus* L.) and chicken in Western UP (India). Bulletin of environmental contamination and toxicology, 79, 396-400.

Lin S.; Pan Y.; Luo L. and Luo L. (2011) Effects of dietary 1, 3-glucan, chitosan or raffinose on the growth, innate immunity and resistance of koi (*Cyprinus carpio* koi). Fish and Shellfish Immunology, 31, 788-94

Lu, Z.Y.; Feng, L.; Jiang, W.D.; Wu, P.; Liu, Y. and Jiang, J. (2021). Mannan oligosaccharides application: multipath restriction from *Aeromonas hydrophila* infection in the skin barrier of grass carp (*Ctenopharyngodon idella*). Front. Immunol. 12, 742107.

Maryam, S.; Naz, H.; Ahmed, T.; Omran, A.; Alanazi, Y. F.; Usman, M. and Heidary, K. (2023). Determination of histological and genotoxic parameters of Nile Tilapia, *Oreochromis niloticus* exposed to lead (Pb). Cellular and Molecular Biology, 69(4), 7-15.

Matović, V.; Buha, A.; Đuki´c-Cosi ´ ´c, D. and Bulat, Z., (2015). Insight into the oxidative stress induced by lead and/or cadmium in blood, liver and kidneys. Food Chem. Toxicol. 78, 130–140.

Mehrim, A.I. and Refaey, M.M. 2023. An overview of the implication of climate change on fish farming in Egypt. Sustainability, 15, 1679.

Mohanty, B. (2024). Prebiotic fructooligosaccharides ameliorate the lipop olysaccharides-induced oxidative stress of mice brain. Journal of Applied Pharmaceutical Science, 14(1), 273-279.

Mondal, P.; Shaw, P.; Bandyopadhyay, A.; Bhowmik, A.D.; Chakraborty, A.; Sudarshan, M. and Chattopadhyay, A. (2019.) Mixture effect of arsenic and fluoride at environmentally relevant concentrations in zebrafish (*Danio rerio*) liver: expression pattern of Nrf2 and related xenobiotic metabolizing enzymes. Aquat. Toxicol. 213, 105219.

Moustafa, E. M.; Farrag, F. A.; Shukry, M.; El-Din, H. M. S. and Omar, A. A. (2024). Ameliorative effect of BIO-MOS® as a dietary supplementation on growth performance, physiological response, oxidative status, and immunity-linked gene expression in Nile tilapia (*Oreochromis niloticus*) fingerlings challenged with *Aeromonas hydrophila*. Open Veterinary Journal, 14(1), 116.

Nawaz, A.; Irshad, S.; Hoseinifar, S. H. and Xiong, H. (2018). The functionality of prebiotics as immunostimulant: Evidences from trials on terrestrial and aquatic animals. Fish and shellfish immunology, 76, 272-278.

Newman, K. (2007). Form follows function in picking MOS product. Feedstuffs, 79: 1-2.

Peterson, B.C.; N.J. Booth; F.T. Barrows and B.B. Manning. (2012). Improved survival in channel catfish fed mannan oligosaccharides in an extruded diet. Open J. Anim. Sci., 2: 57-61.

Rajeshkumar, S.; Liu, Y.; Ma, J.; Duan, H. Y. and Li, X. (2017). Effects of exposure to multiple heavy metals on biochemical and histopathological alterations in common carp, *Cyprinus carpio* L. Fish and shellfish immunology, 70, 461-472.

Ren, Z.; Wang, S.; Cai, Y.; Wu, Y.; Tian, L.; Wang, S.; Jiang, L.; Guo, W.; Sun, Y. and Zhou, Y. (2020). Effects of dietary mannan oligosaccharide supplementation on growth performance, antioxidant capacity, non-specifc immunity and immune-related gene expression of juvenile hybrid grouper (*Epinephelus lanceolatus* ♀ X *Epinephelus fuscoguttatus* ♂). Aquaculture 523, 735195.

Ringø, E.; R.E. Olsen; T.O. Gifstad; R.A. Dalmo; H. Amlund; G.I. Hemre and A.M. Bakke. (2010). Prebiotics in aquaculture: a review. Aquacult. Nutr., 16: 117-136.

Sado R.Y.; Bicudo Á.J.D.A. and Cyrino J.E.P. (2008) Feeding Dietary Mannan Oligosaccharides to Juvenile Nile Tilapia, *Oreochromis niloticus*, Has No Effect on Hematological Parameters and Showed Decreased Feed Consumption. Journal of the World Aquaculture Society, 39, 821-6.

Selim, K. M. and Reda, R. M. (2015). Beta-glucans and mannan oligosaccharides enhance growth and immunity in Nile tilapia. North American Journal of Aquaculture, 77(1), 22-30.

Sharma, R.; Jindal, R.; Jhamb, N.; Banaee, M. and Faggio, C. (2025). Lead Nitrate (Pb (NO3) 2) Toxicity Effects on DNA Structure and Histopathological Damage in Gills of Common Carp (*Cyprinus carpio*). Microscopy Research and Technique, 88(3), 810-817.

Shaw, P.; Mondal, P.; Bandyopadhyay, A. and Chattopadhyay, A., (2019). Environmentally relevant concentration of chromium activates Nrf2 and alters transcription of related XME genes in liver of zebrafish. Chemosphere 214, 35–46.

Shelby R.A.; Lim C.; Yildirim-Aksoy M.; Welker T.L. and Klesius P.H. (2009) Effects of Yeast Oligosaccharide Diet Supplements on Growth and Disease Resistance in Juvenile Nile Tilapia, *Oreochromis niloticus*. Journal of Applied Aquaculture, 21, 61-71.

Singh, N. P.; McCoy, M.T.; Tice ,R.R. and Schneider, L. E. A. (1988): "Simple Technique for Quantitation of low levels of DNA Damage in Individual Cells". Exp.Cell Res., (123):291-298.

Subotić, S.; Spasić, S.; Višnjić-Jeftić, Ž.; Hegediš, A.; Krpo-Ćetković, J.; Mićković, B. and Lenhardt, M. (2013). Heavy metal and trace element bioaccumulation in target tissues of four edible fish species from the Danube River (Serbia). Ecotoxicology and environmental safety, 98, 196-202.

Torrecillas, S.; D. Montero and M. Izquierdo. (2014). improved health and growth of fish fed mannan oligosaccharides: potential mode of action. Fish Shellfish Immunol. 36: 525-544.

Wang, T.; Wu, H. X.; Li, W. J.; Xu, R.; Qiao, F.; Du, Z. Y. and Zhang, M. L. (2022). Effects of dietary mannan oligosaccharides (MOS) supplementation on metabolism, inflammatory response and gut microbiota of juvenile Nile tilapia (*Oreochromis niloticus*) fed with high carbohydrate diet. Fish and Shellfish Immunology, 130, 550-559.

Whittington R.; Lim C. and Klesius P.H. (2005) Effect of dietary glucan levels on the growth response and efficacy of *Streptococcus iniae* vaccine in Nile tilapia, *Oreochromis niloticus*. Aquaculture, 248, 217-25.

Zhai, Q.; Wang, H.; Tian, F.; Zhao, J.; Zhang, H. and Chen, W. (2017). Dietary Lactobacillus plantarum supplementation decreases tissue lead accumulation and alleviates lead toxicity in Nile tilapia (*Oreochromis niloticus*). Aquaculture Research, 48(9), 5094-5103.

Zhang, Q.; Li, L.; Ma, Z.; He, W.; Huang, E.; Meng, L. and Liu, H. (2025). Effects of Mannan Oligosaccharides on Growth, Antioxidant and Immune Performance, and mTOR Signaling Pathway in Juvenile Tilapia (*Oreochromis niloticus*). Animals, 15(16), 2459.