Chronicles of Biomedical Sciences
ISSN:3065-923X
Homepage: https://cbsciences.us/index.php/cbs

Hepatotoxic Effect of Combined Exposure of Arsenic and Microplastics on *Labeo Rohita*: An *In-Vivo* Experimental Study

Saima Yasin[1], Amina Mustafa[1], Ayesha Tariq[1], Munazza Hashim[1], Sania[1], Hira Shahzadi[1], Ghosia Noreen[2], Ayesha Ghafoor[3]

[1]Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan.
[2]Drpartment of Zoology, Government College University (GCU), Lahore, Pakistan.
[3]Department of Zoology, Government College University, Faisalabad, Pakistan.

ORIGINAL ARTICLE

Received on: April 20, 2025.
Accepted on: May 14, 2025.
Published on: May 15, 2025.
Keywords: Arsenic; Hematology; Inflammation; Microplastics; Oxidative stress.

Corresponding author: Ayesha Tariq
tariqayesha912@gmail.com

ABSTRACT

Background: Arsenic (As) is one of major environmental toxicants that adversely affect living organisms. Microplastics (MPs) are small plastic particles that are hazardous because they may be toxic or conjugate with some toxic elements.
Objective: The present research was conducted to assess whether the treatment of As with polystyrene microplastics (PS-MPs) enhance their toxic effects on the hepatic tissues of *Labeo rohita*.
Methods: The experiment was conducted at Government College University, Faisalabad, using 85 healthy *Labeo rohita* (average 8.91 cm, 7.3 g). Fish were acclimatized and randomly divided into four groups i.e., control group, As treatment group ($4.05 mgL^{-1}$), PS-MPs treatment group ($1 mgL^{-1}$), and As ($4.05 mgL^{-1}$) plus PSMPs ($1 mgL^{-1}$) combined treatment group for 21 days. Blood and liver samples were collected post-treatment for hematological, antioxidant, hepatic, and inflammatory analyses. Enzymatic activities and biomarkers were measured using standard biochemical methods.
Results: Exposure to As and PS-MPs, especially their combination, significantly reduced RBCs and hemoglobin, increased WBCs, and suppressed antioxidant enzyme activities (SOD, CAT, GST). Reactive oxygen species (ROS) and Thiobarbituric acid reactive substances (TBARS) levels were elevated, indicating oxidative stress. Liver enzymes (ALT, AST) and inflammatory cytokines (IL-1β, IL-6) were significantly higher in the As+PS-MPs group compared to individual treatments or control.
Conclusion: Co-exposure to As and PS-MPs caused severe hematological, oxidative, hepatic, and inflammatory disturbances in *L. rohita*. Their combined toxicity was greater than individual exposures, indicating a synergistic effect that may lead to pronounced liver damage and physiological dysfunction.

Citation: Yasin S, Mustafa A, Tariq A, Hashim M, Sania, Shahzadi H, et al. Hepatotoxic effect of combined exposure of arsenic and microplastics on *Labeo rohita*: an *in-vivo* experimental study. Chron Biomed Sci. 2025;2(2):52. Available from: https://cbsciences.us/index.php/cbs/article/view/52.

Introduction

Environmental pollution, especially of water, is one of the key global problems. Not only is water pollution seriously impacting on the survival, development and reproduction of aquatic organisms but it also has an influence on the lives of human beings due to its ability of bioaccumulation [1].

Fish is one of the staple foods that is cost-effective, with high nutritional animal protein content [2]. Fish development, especially early development, is quite susceptible to water contamination. Aquatic environment contamination by heavy metals (e.g., lead (Pb), cadmium

(Cd), arsenic (As), mercury (Hg) etc.) significantly affect the development and physiology of fish, including the development of organs, spawning, and reproduction, leading to the reduced offspring quantity and quality [3]. Waterborne heavy metals intake by a fish lead to functional and structural disruptions in numerous tissues as well as organs [4].

Arsenic (As) is one of the key toxicants in the aquatic ecosystem that contaminates water [5]. As is a metalloid toxicant that is found extensively throughout the world in lakes, canals, rivers, ponds, and groundwater, as well as seawater due to the unregulated flow of industrial waste products and pesticides into the aquatic ecosystem [6]. The World Health Organization (WHO) has placed As among the leading chemicals posing risk to public health [7]. As concentrations have been recorded up to 800-2500ppm in numerous countries, i.e., Bangladesh and Chile [8].

High level of As exposure has been directly related to numerous diseases like lung and skin cancers along with cardiovascular diseases and liver disorders [9]. The adverse effects of As on fish growth, development, RNA/DNA ratio, histopathology, gene expression and mortality have been established through earlier studies [10]. This toxicant is capable of inducing biochemical as well as physiological alterations in the fish that produce harmful effects in the development as well as growth of fish [11]. Elevated concentrations of As alters the fish physiology and result in immune and reproductive disorder and even death [12].

Microplastics (MPs) are categorized into primary and secondary MPs depending on where they originate from and the manufacturing process [13]. The primary MPs are small pieces of plastic that are generally used in textiles, care products, and drugs. These particles enter aquatic environments primarily via runoff from the surface waters and discharge from treatment plants (WWTPs) [14]. The larger pieces of plastic degrade into secondary MPs. Some of the major sources of these large pieces are fishing nets, resin materials, domestic products, and disposable products [8][15]. The process of breakdown raises the overall environmental concentration of MPs, which is becoming a global concern.

MPs affect the health of humans because they have the ability to accumulate in seafood [16]. Aquatic organisms ingest MPs that bioaccumulate in certain organs, leading to oxidative stress, thus decreasing the growth and development of sea animals [13]. MPs have adverse effects on the health of living organisms and damages various organs, such as gut, liver, and kidney, testes [17][18][19][20]. When MPs are consumed by fishes, they are deposited in the organs and tissues of fishes, leading to different kinds of negative health effects [21]. MPs ingestion causes severe damage to the liver, including enhanced accumulation of fats, inflammation, fibrosis, structural injury, and apoptosis [22].

Oxidative stress is an occurrence where the body's reactive oxygen species (ROS) are unbalanced compared to antioxidants scavenging them [23]. ROS are naturally occurring or induced molecules produced within the body through metabolic activities, but they can be elevated by external stimuli like exposure to pollutants or toxicants. Excess ROS can bring about harm to cells and tissues, resulting in inflammation as well as other negative health outcomes. Reducing the nutrition level of the fish as well as the fish population has an important influence on the diet of humans but also on tradition and economy globally [24].

As and MPs are among the most prevalent environmental pollutants. Research suggests that MPs may increase the bioaccumulation of other toxicants as they can attach with MPs. After attaching with MPs, these chemicals not only accumulate in the body, but their adverse effects are also increased significantly [25]. Therefore, the current research was conducted to assess the combined effect of MPs and As on the hepatic tissues of *Labeo rohita*. We hypothesized that MPs may enhance the toxicity of As and induce toxicity in the liver of *L. rohita*.

Methods

Experimental Animals

This *in-vivo* experimental study was executed in Government College University, Faisalabad, Pakistan. 85 healthy and uniform sized *L. rohita* were purchased from local hatchery and transported to the research site. The fish were 90 days old, and their average length and weight were 8.91±1.32cm and 7.3±1.57g, respectively. The fish was kept in glass aquariums and acclimatized in the laboratory environment for 90 days at 25±1℃ temperature, 6.0-7.5 mg L^{-1} dissolved oxygen (DO) and 7.5-8.5 pH. The fish were treated with 5g/L sodium chloride to lower the risk of parasites and infection. Fish were given commercial feed and the ethical guidelines of EU (2010/63/EU) were followed during the experiment.

Chemicals

Polystyrene microplastics (PS-MPs) and arsenic (As) were purchased from Macklin Biochemical Technology (Shanghai, China). All other chemicals used in the experiment were of analytical grade.

Group allocation

Fish were kept randomly assorted into 4 equal groups and each group contained 20 fish. The groups included, Control, As treated (4.05mgL^{-1}), PS-MPs treated (1mgL^{-1}) and As (4.05mgL^{-1})+PSMPs (1mgL^{-1}) co-treated group.

The experiment was conducted for 21 days and then the fish were anaesthetized and killed. Their blood was taken in blood vials and stored at -20℃ for subsequent analysis. Moreover, their liver was taken out and placed in ice box and kept at -20℃ for biochemical analysis.

Biochemical tests

Biochemical tests were conducted to measure the concentrations of blood parameters, antioxidant enzymes, inflammatory indices and liver function markers. DxH 900 Hematology Analyzer (Beckman Coulter, California, USA) was used to determine hematology parameters including red blood cells (RBCs) count, white blood cells (WBCs) count, and Hemoglobin concentrations.

Hepatic tissues were homogenized and supernatant was collected and stored at -80℃ for further analysis. The activities of antioxidant enzymes i.e., SOD, GST and CAT were estimated via the methodology of Winterbourn et al. [26], Habig et al. [27] and Aebi et al. [28], respectively. Moreover, the levels of Thiobarbituric acid reactive substances (TBARS) and ROS were estimated as per the techniques of Iqbal et al. [29] and Hayashi et al., [30], respectively.

The activity of ALT, AST and ALP was estimated by using the kits provided by Bioactive Diagnostic (Bad Homburg, Germany). Moreover, the levels of inflammatory cytokines (IL-1β, and IL-6) were measured using ELISA kits (Cusabio, US).

Statistical analysis

The data were expressed as Mean±SEM. SPSS (v26) was used for the statistical analysis and GraphPad Prism 9 was used to generate graphs. One-way ANOVA followed by Tukey's test was employed for the comparison of groups. P value less than 0.05 was considered significant.

Results

Effects of exposure of As and PS-MPs on hematological markers

The results in Table 1 showed that As and PS-MPs exposure significantly affected the concentration of hematological markers. RBCs and hemoglobin were significantly ($p<0.05$) reduced and WBCs were increased after the exposure to As, PS-MPs and As + PS-MPs co-exposure, as compared to control. Moreover, RBCs, WBCs and hemoglobin exhibited non-significant differences among As and PS-MPs group. However, As + PS-MPs co-treated group was affected the most and showed lowest concentrations of RBCs and hemoglobin and high concentrations of WBCs, as compared to the other groups.

Table 1. The effect of As and PS-MPs on hematological markers.

	Control	As	PS-MPs	As + PS-MPs
RBC	3.31 ± 0.07^{a}	2.79 ± 0.06^{b}	2.68 ± 0.09^{b}	1.94 ± 0.10^{c}
WBC	12.31 ± 0.19^{c}	18.93 ± 0.38^{b}	17.56 ± 0.48^{b}	24.62 ± 0.87^{a}
Hb	9.13 ± 0.22^{a}	7.83 ± 0.21^{b}	8.01 ± 0.31^{b}	5.28 ± 0.14^{c}

RBC (10^6/mm^3), WBC (10^3/mm^3), Hemoglobin (g/dL). Values showing different superscripts are significantly ($p<0.05$) different from each other.

Effects of exposure of As and PS-MPs on antioxidant enzymes and oxidative stress markers

Figure 1 and 2 shows the effect of As and PS-MPs on antioxidant enzymes and oxidative stress markers. The graphs show that As, PS-MPs and As + PS-MPs exposure significantly ($p<0.05$) reduced the activities of SOD, CAT and GST while increasing the level of TBARS and ROS, as compared to the control group. However, the levels of these markers were approximately similar in the As and PS-MPs treated groups. Nevertheless, the group co-treated with As + PS-MPs showed the lowest activities of antioxidants and the highest levels of TBARS and ROS.

Effects of exposure of As and PS-MPs on hepatic serum and inflammatory markers

Figure 3 and 4 demonstrate that As and PS-MPs exhibited marked impact on the levels of hepatic serum and inflammatory markers. The levels of ALT, AST, IL-1β and IL-6 were significantly ($p<0.05$) increased following As, PS-MPs and As + PS-MPs treatment, as matched with the control. However, the differences in the levels of these indices were insignificant in the As and PS-MPs treated groups. However, As + PS-MPs co-exposed group exhibited the highest levels of these markers that were significantly ($p<0.05$) different from the other groups. This suggests that As + PS-MPs co-treated group was most the affected one.

Discussion

The level of As content of natural water bodies is largely dependent on geological composition, as well as the level of pollution [31]. The results of current experiment revealed that As and PS-MPs exposure reduced RBCs count and hemoglobin concentration along with increasing WBCs count. Pedlar et al., [32] reported that As exposure produced anemic conditions in the fish. It has been documented that, heavy metal exposure decreased the number of RBCs in fish [33].

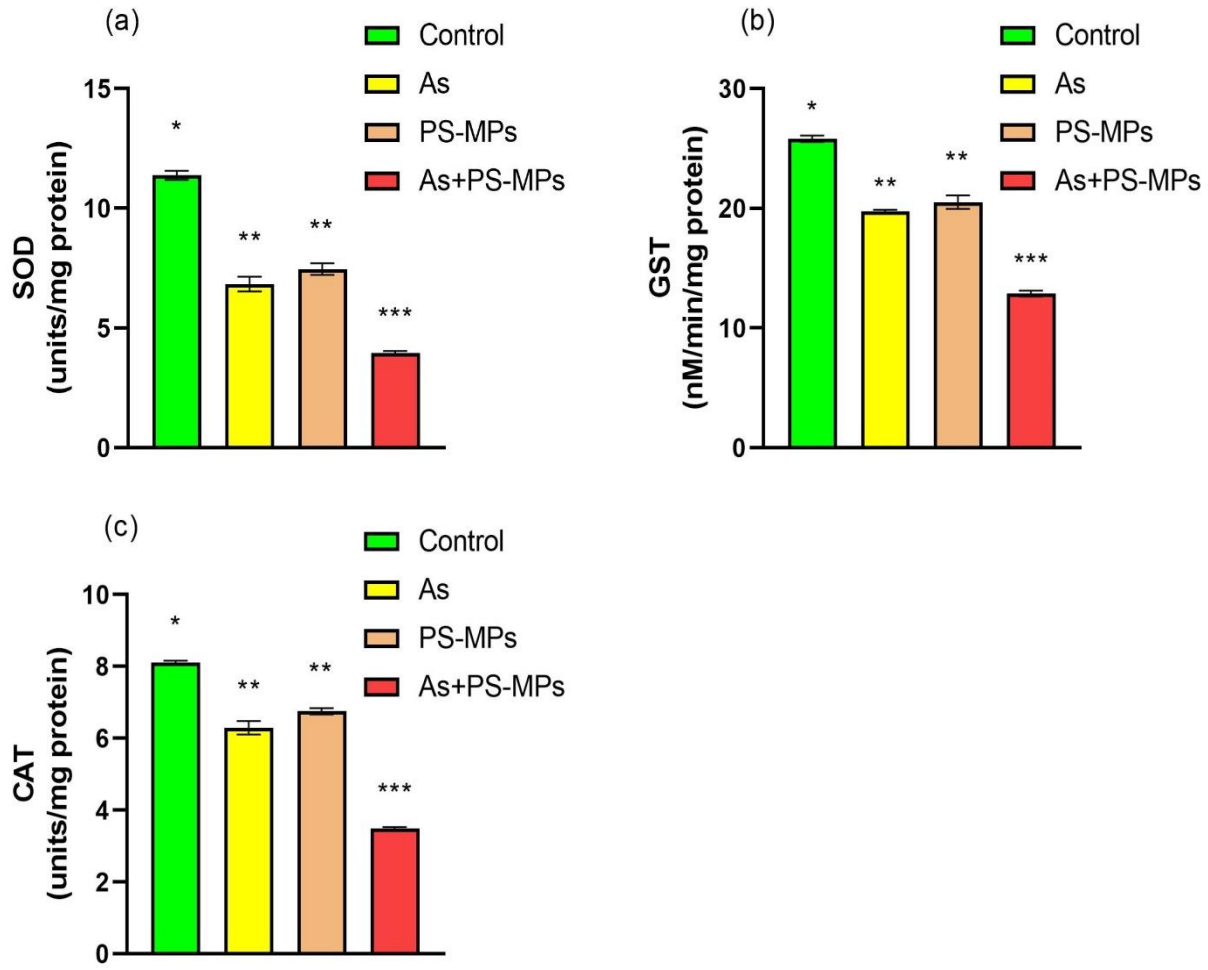

Figure 1: Effect of As and PS-MPs are shown on (a) SOD, (b) GST and (c) CAT. Different superscripts above the bar show that significant differences are present.

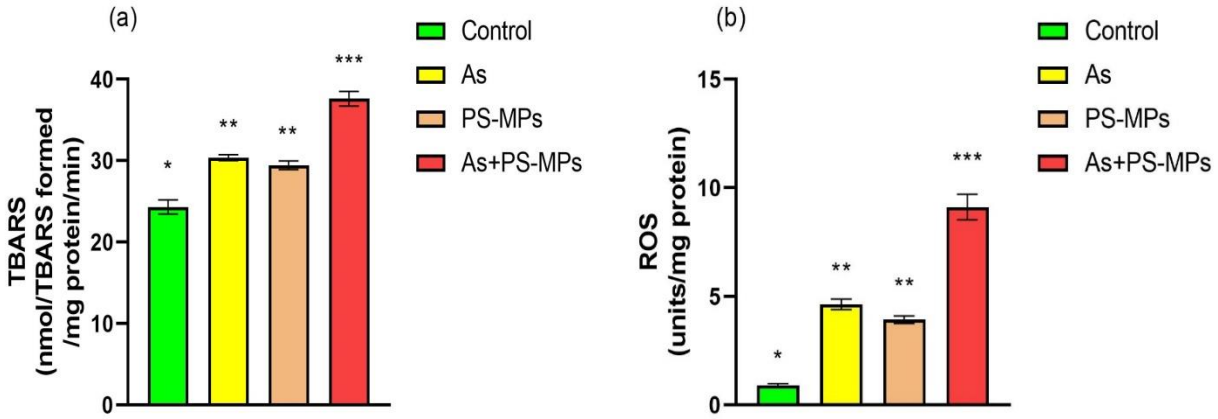

Figure 2: Effect of As and PS-MPs are shown on (a) TBARS, and (b) ROS. Different superscripts above the bar show that significant differences are present.

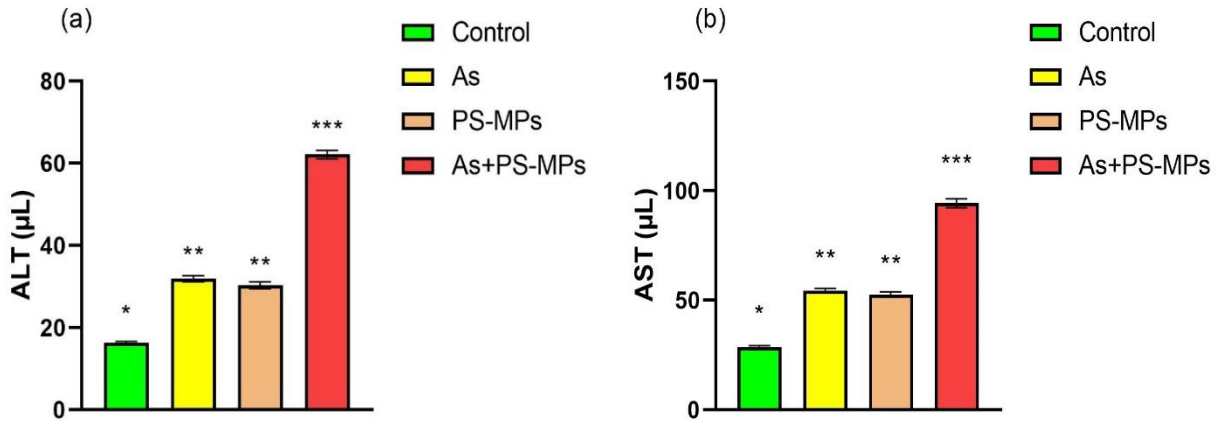

Figure 3: Effect of As and PS-MPs are shown on (a) ALT, and (b) ALP. Different superscripts above the bar show that significant differences are present.

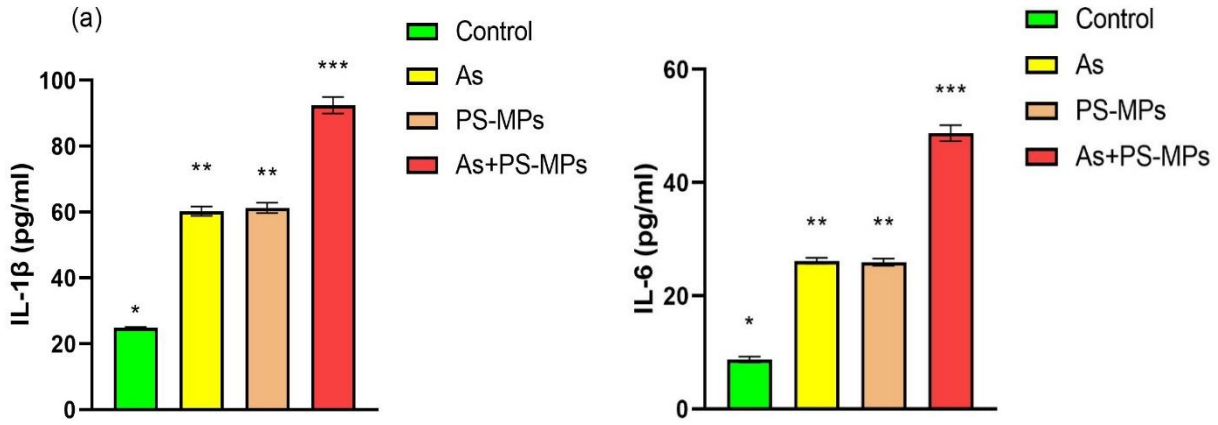

Figure 4: Effect of As and PS-MPs are shown on (a) IL-1β, and (b) IL-6. Different superscripts above the bar show that significant differences are present.

Moreover, As exposure also reduced the levels of hemoglobin and packed cell volume of *L. rohita* [34]. Leucocytes are engaged in the modulation of immunological function within most organisms and the WBC increase of stressed animals signifies a protective reaction towards stress [35]. The significantly decreased levels of RBCs, and hemoglobin and increased WBCs in As+PS-MPs co-treated group show that it deleteriously impacted the hematological parameters of fish.

Exposure to As and PS-MPs decreased the antioxidant activities and increased the levels of TBARS and ROS. SOD, and CAT are three key enzymes of first line defense mechanism that directly assists to degrade harmful ROS. Enzyme SOD acts through catalyzing the dismutation of toxic superoxide anion (O^{2-}) to molecular oxygen (O_2) and less toxic hydrogen peroxide (H_2O_2). This H_2O_2 is removed subsequently by the combined action of CAT and glutathione peroxidase (GPx). These enzymes acts through catalyzing reduction of H_2O_2 to harmless products. GPx can also act against other peroxides [36]. GST is another

antioxidant enzyme which is involved in detoxification and neutralization of ROS [37]. Method of assessing the oxidative stress levels have been extensively used in studies on mechanisms of environmental toxicity as well as ecotoxicity towards living organisms under exposure of contaminants [38]. Oxidative stress could be quantified directly by the production of free radicals, indirectly by the defense against the reactive species by antioxidants and by determining the end-products of oxidative damage [39]. TBARS are the markers of lipid peroxidation and oxidative stress [40]. It was revealed that As and PS-MPs co-exposure produced enhanced toxic effects as evidenced by significantly compromised antioxidant levels and increased ROS and TBARS levels.

In the current research, exposure to As and PS-MPs increased the levels of hepatic function markers. Moreover, ALT, and AST enzymes are present in maximum number of fish organs including heart, skeletal muscle, kidney, pancreas, spleen, erythrocyte, brain, liver and gill [41]. Upon damage of these tissues or cells, particularly of liver through disease or injury, AST as well as ALT are released and subsequently invade into the blood stream [42]. The notably higher levels of liver markers in As + PS-MPs co-exposed group showed that they significantly impacted the hepatic function and damaged the liver.

Our results indicated that as a result of AS and PS-MPs exposure, there was a rise in pro-inflammatory cytokine levels (IL-1β, and IL-6), which resulted in hepatic toxicity in *L. rohita*. Thus, upregulation of pro-inflammatory cytokines can be supplemented with a rise in anti-inflammatory cytokine levels to trigger the immune response as well as keep the microenvironment homeostatic [43]. IL-1β is triggered in the condition of organ damage, pain and inflammation and it is a marker of inflammatory response [44]. Moreover, IL-6 is another marker of inflammation and its elevated levels are found in case of inflammation due to multiple reasons, including metabolic diseases or toxicity [45]. It was revealed that, PS-MPs and As co-exposure upsurged the levels of IL-1β, and IL-6, showing marked inflammation in the hepatic tissues of *L. rohita.*

Conclusion: In conclusion, As and PS-MPs co-exposure significantly damaged the hepatic tissues of *L. rohita.* Their combined treatment enhanced the hazardous effects of As and lead to hematological impairments in the fish. Moreover, they also disturbed the activities of antioxidant enzymes and induced oxidative stress. Additionally, PS-MPs and As co-exposure increased the levels of hepatic function markers and inflammatory markers. Therefore, As and PS-MPs co-treatment may cause hepatic damage in *L. rohita* due to their ability to cause oxidative stress and inflammation in the body of fish.

Authors' contributions

ICMJE criteria	Details	Author(s)
1. Substantial contributions	Conception, OR Design of the work, OR Data acquisition, analysis, or interpretation	1,8 2,5,7 3,4,6
2. Drafting or reviewing	Draft the work, OR Review critically for important intellectual content	1,2,5,6 3,4,7,8
3. Final approval	Approve the version to be published	1,2,3,4, 5,6,7,8
4. Accountable	Agree to be accountable for all aspects of the work	1,2,3,4, 5,6,7,8

Acknowledgement
None

Funding
This research study received no specific grant from any funding agency.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations
Ethics approval and consent to participate
The Ethics Review Committee of Government College University, Faisalabad approved the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

References

[1]. Dai YJ, Jia YF, Chen N. Zebrafish as a model system to study toxicology. Environ Toxicol Chem. 2014;33:11-7. doi:10.1002/etc.2406

[2]. Kumari B, Kumar V, Sinha AK. Toxicology of arsenic in fish and aquatic systems. Environ Chem Lett. 2017;15:43-64. doi:10.1007/s10311-016-0588-9

[3]. Eerkes-Medrano D, Thompson RC, Aldridge DC. Microplastics in freshwater systems: a review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. Water Res. 15;75:63-82. doi:10.1016/j.watres.2015.02.012

[4]. Maulu S, Nawanzi K, Abdel-Tawwab M, Khalil HS. Fish nutritional value as an approach to children's nutrition. Front Nutr. 15;8:780844. doi:10.3389/fnut.2021.780844
[5]. Babich R, Van Beneden RJ. Effect of arsenic exposure on early eye development in zebrafish (Danio rerio). J Appl Toxicol. 2019;39:824-31. doi:10.1002/jat.3770
[6]. Naujokas MF, Anderson B, Ahsan H, Aposhian HV, Graziano JH, Thompson C, et al. The broad scope of health effects from chronic arsenic exposure: update on a worldwide public health problem. Environ Health Perspect. 2013;121:295-302. doi:10.1289/ehp.1205875
[7]. Bräuner EV, Nordsborg RB, Andersen ZJ, Tjønneland A, Loft S, Raaschou-Nielsen O. Long-term exposure to low-level arsenic in drinking water and diabetes incidence: a prospective study of the diet, cancer and health cohort. Environ Health Perspect. 2014;122:1059-65. doi:10.1289/ehp.1408198
[8]. Han JM, Park HJ, Kim JH, Jeong DS, Kang JC. Toxic effects of arsenic on growth, hematological parameters, and plasma components of starry flounder, Platichthys stellatus, at two water temperature conditions. Fish Aquat Sci. 2019;22:1-8. doi:10.1186/s41240-019-0116-5
[9]. Jezierska B, Witeska M. Metal toxicity to fish. Monografie. University of Podlasie (Poland). 2001(42).
[10]. Foley CJ, Bradley DL, Höök TO. A review and assessment of the potential use of RNA: DNA ratios to assess the condition of entrained fish larvae. Ecol Indic. 2016;60:346-57. doi:10.1016/j.ecolind.2015.07.005
[11]. Li J, Liu H, Chen JP. Microplastics in freshwater systems: A review on occurrence, environmental effects, and methods for microplastics detection. Water Res. 2018;137:362-74. doi:10.1016/j.watres.2017.12.056
[12]. Ahmed MK, Habibullah-Al-Mamun M, Parvin E, Akter MS, Khan MS. Arsenic induced toxicity and histopathological changes in gill and liver tissue of freshwater fish, tilapia (Oreochromis mossambicus). Exp Toxicol Pathol. 2013 Sep 1;65:903-9. doi:10.1016/j.etp.2013.01.003
[13]. Ratnaike RN. Acute and chronic arsenic toxicity. Postgraduate medical journal. 2003;79:391-6. doi:10.1136/pmj.79.933.391
[14]. Gall SC, Thompson RC. The impact of debris on marine life. Mar Pollut Bull. 2015;92:170-9. doi:10.1016/j.marpolbul.2014.12.041
[15]. Kozioł A, Paso KG, Kuciel S. Properties and recyclability of abandoned fishing net-based plastic debris. Catalysts. 2022;12:948. doi:10.3390/catal12090948
[16]. Burgos-Aceves MA, Faggio C, Betancourt-Lozano M, González-Mille DJ, Ilizaliturri-Hernández CA. Ecotoxicological perspectives of microplastic pollution in amphibians. J Toxicol Environ Health B Crit Rev. 2022;25:405-21. doi:10.1080/10937404.2022.2140372
[17]. Nazir N, Akbar A, Salar MZ, Ahmed MZ, Ishtiaq A. Pharmacological assessment of delphinidin in counteracting polystyrene microplastic induced renal dysfunction in rats. J King Saud Univ Sci. 2024;36:103462. doi:10.1016/j.jksus.2024.103462
[18]. Ghafoor N, Mehar T, Batool M, Salar MZ, Ahmed MZ, Atique U. Attenuative effects of poncirin against polyethylene microplastics-prompted hepatotoxicity in rats. J King Saud Univ Sci. 2024;36:103475. doi:10.1016/j.jksus.2024.103475
[19]. Alvi K, Hamza A, Ehsan N, Salar MZ, Ahmed Z, Atique U. Juglanin cures polyethylene microplastics-induced testicular damage in rats. J King Saud Univ Sci. 2024;36:103394. doi:10.1016/j.jksus.2024.103394
[20]. de Oliveira RB, Pelepenko LE, Masaro DA, Lustosa GM, de Oliveira MC, Roza NA, et al. Effects of microplastics on the kidneys: A narrative review. Kid Int. 2024;106(3):400-7. doi:10.1016/j.kint.2024.05.023
[21]. Liu Y, Jia X, Zhu H, Zhang Q, He Y, Shen Y, et al. The effects of exposure to microplastics on grass carp (Ctenopharyngodon idella) at the physiological, biochemical, and transcriptomic levels. Chemosphere. 2022;286:131831. doi:10.1016/j.chemosphere.2021.131831
[22]. Elsheikh AA, Alnasser SM, Shalaby AM, Alabiad MA, Abd-Almotaleb NA, Alorini M, et al. Polystyrene microplastic particles induced hepatotoxic injury via pyroptosis, oxidative stress, and fibrotic changes in adult male albino rats; the therapeutic role of silymarin. Toxicol Mech Methods. 2023;33(6):512-28. doi:10.1080/15376516.2023.2191270
[23]. Bhatti FU, Kim SJ, Yi AK, Hasty KA, Cho H. Cytoprotective role of vitamin E in porcine adipose-tissue-derived mesenchymal stem cells against hydrogen-peroxide-induced oxidative stress. Cell Tissue Res. 2018;374:111-20. doi:10.1007/s00441-018-2857-3
[24]. Liang W, Li B, Jong MC, Ma C, Zuo C, Chen Q. Process-oriented impacts of microplastic fibers on behavior and histology of fish. J Hazard Mat. 2023;448:130856. doi:10.1016/j.jhazmat.2023.130856
[25]. Jia H, Wu D, Yu Y, Han S, Sun L, Li M. Impact of microplastics on bioaccumulation of heavy metals in rape (*Brassica napus* L.). Chemosphere, 2022;288:132576. doi:10.1016/j.chemosphere.2021.132576
[26]. Winterbourn CC, Hawkins RE, Brian M, Carrell RW. The estimation of red cell superoxide dismutase activity. J Lab Clin Med. 1975;85:337-41.
[27]. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J Biol Chem. 1974;249:7130-9. doi:10.1016/S0021-9258(19)42083-8
[28]. Aebi H, Mörikofer-Zwez S, von Wartburg JP. Alternative molecular forms of erythrocyte catalase. InStructure and Function of Oxidation–Reduction Enzymes 1972 (pp. 345-351). Pergamon. doi:10.1016/B978-0-08-016874-6.50044-8
[29]. Iqbal M, Sharma SD, Rezazadeh H, Hasan N, Abdulla M, Athar MJ. Glutathione metabolizing enzymes and oxidative stress in ferric nitrilotriacetate mediated hepatic injury. Re-

dox Rep. 1996;2:385-91. doi:10.1080/13510002.1996.11747079
[30]. Hayashi I, Morishita Y, Imai K, Nakamura M, Nakachi K, Hayashi T. High-throughput spectrophotometric assay of reactive oxygen species in serum. Mut Res. 2007;631:55-61. doi:10.1016/j.mrgentox.2007.04.006
[31]. Jain CK, Ali I. Arsenic: occurrence, toxicity and speciation techniques. Water Res. 2000;34:4304-12. doi:10.1016/S0043-1354(00)00182-2
[32]. Pedlar RM, Ptashynski MD, Evans R, Klaverkamp JF. Toxicological effects of dietary arsenic exposure in lake whitefish (Coregonus clupeaformis). Aquat Toxicol. 2002;57:167-89. doi:10.1016/S0166-445X(01)00198-9
[33]. Chowdhury MJ, Pane EF, Wood CM. Physiological effects of dietary cadmium acclimation and waterborne cadmium challenge in rainbow trout: respiratory, ionoregulatory, and stress parameters. Comp Biochem Physiol C: Toxicol Pharmacol. 2004;139:163-73. doi:10.1016/j.cca.2004.10.006
[34]. Tripathi S, Sahu DB, Kumar R, Kumar A. Effect of acute exposure of sodium arsenite (Na3 Aso3) on some haematological parameters of *Clarias batrachus* (common Indian cat fish) in vivo. Indian J Environ Health. 2003;45:183-8.
[35]. Nussey G, Van Vuren JH, Du Preez HH. Effect of copper on blood coagulation of Oreochromis mossambicus (Cichlidae). Comp Biochem Physiol C: Pharmacol Toxicol Endocrinol. 1995;111:359-67. doi:10.1016/0742-8413(95)00062-3
[36]. Kotsanis N, Iliopoulou-Georgudaki J, Kapata-Zoumbos K. Changes in selected haematological parameters at early stages of the rainbow trout, Oncorhynchus mykiss, subjected to metal toxicants: arsenic, cadmium and mercury. J Appl Ichthyol. 2000;16:276-8. doi:10.1046/j.1439-0426.2000.00163.x
[37]. Kim Y, Cha SJ, Choi HJ, Kim K. Omega class glutathione S-transferase: antioxidant enzyme in pathogenesis of neurodegenerative diseases. Oxid Med Cell Longev. 2017:5049532.doi:10.1155/2017/5049532
[38]. Halliwell B, Gutteridge JM. Free radicals in biology and medicine. Oxford university press; 2015.
[39]. Faggio C, Pagano M, Alampi R, et al. Cytotoxicity, haemolymphatic parameters, and oxidative stress following exposure to sub-lethal concentrations of quaternium-15 in Mytilus galloprovincialis. Aquat Toxicol. 2016;180:258-65. doi:10.1016/j.aquatox.2016.10.010
[40]. González-Arostegui LG, Cerón JJ, Gök G, Neselioglu S, Erel O, Rubio CP. Validation of assays for measurement of oxidant compounds in saliva of pigs: Thiobarbituric acid reactive substances (TBARS), carbonyl, and reactive oxygen species (ROS). Res Vet Sci. 2023;165:105069. doi:10.1016/j.rvsc.2023.105069
[41]. Srivastava S, Sinha R, Roy D. Toxicological effects of malachite green. Aquat Toxicol. 2004;66:319-29. doi:10.1016/j.aquatox.2003.09.008
[42]. Lala V, Zubair M, Minter D. Liver function tests. StatPearls. 2023. Available from. https://www.statpearls.com/point-of-care/20995/
[43]. Reynolds B, Richards JB, de Wit H. Acute-alcohol effects on the Experiential Discounting Task (EDT) and a question-based measure of delay discounting. Pharmacol Biochem Behav. 2006;83:194-202. doi:10.1016/j.pbb.2006.01.007
[44]. Ren K, Torres R. Role of interleukin-1β during pain and inflammation. Brain Res Rev. 2009;60:57-64. doi:10.1016/j.brainresrev.2008.12.020
[45]. Hirano T. IL-6 in inflammation, autoimmunity and cancer. Int Immunol. 2021;33:127-48. doi:10.1093/intimm/dxaa078