

Article

Ingestion of Polyvinylchloride Powder Particles Induces Oxidative Stress and Hepatic Histopathological Changes in *Oreochromis niloticus* (Nile Tilapia)—A Preliminary Study

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Simple Summary: Plastic debris is categorized by size as macro-, meso-, micro-, and nanoparticles. However, little attention has been given to plastic particles that are invisible to the naked eye, yet too big to be considered nanoparticles. Although the health and ecological effects of the ingestion of micro- and nanoplastics are starting to be well-documented, plastic particles at the lower size limit of microplastics have different characteristics than large (1–5 mm) microplastic particles and may induce higher toxicity. Within our study, we exposed fish specimens to powder particles of polyvinyl chloride (PVC) via ingestion. The polymer used, although considered biologically inert, manifested notable negative effects in its powder form.

Abstract: Plastic debris is considered an emerging aquatic pollutant as an alarming number of reports are indicating the environmental contamination with such agents. Ichthyofauna has been subjected to increasing plastic pollution over the past years, which has led to detrimental effects in the food chain, and consequently to the general health of ecosystems. In this study, we exposed juvenile specimens of *Oreochromis niloticus* to polyvinyl chloride (PVC) in powder form. Specimens and water parameters were closely monitored for 40 days before tissue samples were collected for histological and biochemical analysis. Structural hepatic alterations were observed in specimens from the exposed groups, such as intercellular corridors, dilation of sinusoidal capillaries, hyperchromatic nuclei, nuclear hypertrophy, and cytoplasm vacuolization. Low catalase activity was observed in the case of 1000 mg of PVC/kg feed group, as well as high levels of malondialdehyde compared to the control group, indicating oxidative stress. Glutathione peroxidase activity was also significantly decreased in the 500 and 1000 mg/kg feed group compared to the control group. These findings suggest that a midterm exposure to PVC particles can significantly affect the activity of antioxidative enzymes in *O. niloticus* specimens and induce changes of hepatic tissue structure.

Keywords: microplastics; polyvinyl chloride; oxidative stress; fish; pollutants



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1. Introduction

Plastic waste is now considered a ubiquitous pollutant in both aquatic and terrestrial environments [1,2]. There are different groups of plastic debris that vary in shape,

size, and/or chemical composition, and these characteristics can be related to their toxicity [3,4]. From a chemical point of view, most consumers' plastics can be divided into seven groups [5], among them being polyvinyl chloride (PVC), a polymer produced by polymerization of the toxic vinyl chloride monomer [6]. Although composed of up to 57% vinyl chloride monomers, PVC is the third most manufactured plastic in the world and one of the most widely used plastic materials in everyday items as well as in medical products, being considered safe, versatile, and having a low cost of ownership [7,8], but being highly problematic as a pollutant [9–11].

Despite the fact that most plastic products are considered safe and are an integral part of our everyday lives, [6] plastic waste has high detrimental effects on the ecosystem and public health [12]. It is not only the chemical composition and substances resulting from their degradation, but also the size and shape of the plastic litter itself that causes the detrimental effects [13–15]. Judging by their size, plastic fragments have been divided in nano-, micro-, meso-, macro-, and megasize ranges, but a consensus on the ranges' limits has not been reached [16]. The National Oceanic and Atmospheric Administration concluded that the most prevalent form of plastic litter is microplastics (MPs), which should be defined as fragments <5 mm with a suggested lower size boundary of 333 µm for ease of detection [17]. Others reported MPs as particles with sizes of 1–1000 µm [18] or 1–5000 µm [16], although a clear nomenclature is lacking [18]. Currently, the classes of plastic waste that are receiving the most attention are macro- and microparticles visible by eye, as these can be easily observed in the environment and intestinal tract of wild fauna that mistakenly ingested them as food [19–22].

Consumption of these particles can generally cause mechanical damage or obstruction of the digestive tract, which in turn leads to the prevention of proper nutrient absorption. However, there is a possibility that small microplastics are able to penetrate through the intestinal wall and be transported to other tissues [21], as well as having different size-dependent toxicity [14,23]. The lack of a clear size nomenclature and no general consensus regarding plastic debris size categories is detrimental to their study, as confusion can take place and some plastic debris size classes and their effects can be overlooked or disregarded.

This study focused on PVC particles invisible to the naked eye that are still too big to be considered in the nanosize category (1 nm–1000 nm) [24,25]. PVC is a high-density polymer, a property which leads to it sinking to the bottom of water bodies, thus increasing its prevalence in sediments and therefore, facilitating more PVC ingestion by bottom dwelling species [26]. The species used in this study, *Oreochromis niloticus*, represents a fitting biological model for microplastics toxicity studies through its high economic potential, wide geographic distribution, and ease of brooding in natural and laboratory environments, as well as being a known model to study the responses and adaptations of fish populations to aquatic pollutants in fresh and brackish water [27–31].

The study aimed at gauging the effects of ≈ 50 µm Ø PVC particles on *O. niloticus* via liver histological observations and liver enzymatic activities, as well as levels of lipid peroxidation after a 40-day monitored exposure to the pollutant. The exposure took place via ingestion in a controlled environment, as the species is susceptible to MPs contamination via ingestion, but also via non-ingestion exposure through gills or even tegument [29,32].

2. Materials and Methods

2.1. Specimens and Exposure Conditions

Commercially available juvenile specimens of *Oreochromis niloticus* (length ≈ 6 cm; weight ≈ 4 g), hatched and grown in an experimental facility under a controlled and nontoxic environment, were randomly distributed into 3 groups (10 specimens per group per water tank), using water tanks of 40 L capacity each (Aquamedic nursery water tanks, Aquamedic, Bissendorf, Germany). This study was run with (i) a control group, represented by fish which received feed without PVC, (ii) a second group exposed to 500 mg of PVC per kg feed, and (iii) the third group exposed to 1000 mg of PVC per kg feed. The water tanks with exposed specimens and the water tank with the control group were subjected

to identical water filtration conditions. Water was recirculated constantly via continuous pumping (Oceanrunner 2500, Aquamedic, Bissendorf, Germany) and the filtration was assured by an multilayer sponge bed, activated charcoal, and ceramic beads systems.

Temperature, pH, and oxygen saturation sensors (Aquamedic, Bissendorf, Germany) were connected to the aquariums via a PC platform (AT-Control v7.0.0.6 software) to monitor the parameterst twice per day, before and after feeding; The measurements took place during the 40-day exposure study (Supplementary Table S1), after a 2-week accommodation period.

Feed pellets containing PVC were made in two concentrations: 500 mg of PVC per kg of feed and 1000 mg of PVC per kg of feed, respectively. The feed was prepared by grinding “Aller Futura 0.5–1 mm” (Aller Aqua, Denmark, Christiansfeld) feed pellets (Table 1) and then adding PVC (Polyvinyl chloride powder, Carl Roth, Karlsruhe, Germany) (particles size $\approx 50 \mu\text{m}$ Ø) and water under constant mixing. The paste obtained was reformatted into $\approx 1 \text{ mm}$ Ø pellets using a pellet maker and left to dry on paper towels for 24 h at room temperature. The control group received the same reformatted feed, but without PVC. All specimens were fed once a day with pellets weighing 1.5% of the groups’ total body mass. The specimens were sacrificed by decapitation, as using asphyxiation or other means would have interfered with the enzymatic assays.

Table 1. Nutrient composition of feed pellets used. Nitrogen-free extract (NFE) consists of carbohydrates, sugars, starches, and a major portion of materials classed as hemicellulose in feeds. When crude protein, fat, water, ash, and fiber are added and the sum is subtracted from 100, the difference is NFE.

Category	Unit of Measure
Crude protein (%)	60
Crude fat (%)	15
Nitrogen-free extract (NFE) (%)	5.7
Ash (%)	12.6
Fiber (%)	0.7
P (%)	1.4
Gross energy (MJ)	21.2
Digestible energy (MJ)	19.7

The study was performed in conformity with the Guide for the Use and Care of Laboratory Animals recommendations regarding reduction of animal suffering and number of animals sacrificed [33], being approved by the Ethics Committee of Research within the University of Bucharest (decision no. 42/07.07.2021).

2.2. Biochemical Assays

2.2.1. Preparation of Tissue Homogenates, Catalase (CAT), and Glutathione Peroxidase (GPx)

Liver tissue samples from 5 specimens per group were suspended 1:10 *w/v* in ice-cold buffer (0.1 M Tris-HCl, 5 mM EDTA, pH 7.4) and homogenized for 2 min at 30 movements/sec using a Mixer Mill (MM 301 homogenizer, Retsch, Haan, Germany). The mix was centrifuged at $10,000 \times g$ for 10 min at 4 °C and the supernatant was collected and used for the assays.

CAT activity was measured spectrophotometrically at 240 nm by evaluating the disappearance of H_2O_2 from the reaction medium which also contained total protein extract and 0.1 M potassium phosphate buffer (pH~7.1). The activity of GPx was evaluated by monitoring the oxidation of NADPH by t-butyl hydroperoxide at 340 nm. All enzymatic activities were measured on Specord200Plus spectrophotometer (Analytik Jena, Germany)

and calculated as specific activities (units/mg of protein), the results were expressed as mean values.

2.2.2. Lipid Peroxidation

For monitoring lipid peroxidation under the form of malondialdehyde (MDA), thiobarbituric acid (TBA) was used as the reactive substance. MDA-TBA adducts, formed as a result of the reaction between MDA from the biological sample and TBA at 37 °C, can be measured fluorometrically (λ excitation = 520 nm, λ emission = 549 nm). For 200 μ L of sample with a protein concentration of 1 mg/mL, 700 μ L of 0.1 N HCl was added, and the mixture was incubated for 20 min at room temperature. Then, 900 μ L of 0.025 M thiobarbituric acid was added, and the mixture was incubated for 65 min at 37 °C. The fluorescence was recorded using a Jasco FP750 spectrofluorometer (Tokyo, Japan) with the wavelengths set at 520 nm/549 nm for excitation/emission. MDA content was calculated based on a 1,1,3,3-tetramethoxypropane standard curve in the range of 0–0.5 μ M. The results were expressed as nmoles of MDA/mg protein.

2.3. Histological Analysis

Liver samples were immediately collected after tissue clarification and briefly kept on ice prior to being immersed in the fixative solution (25 mL of formaldehyde, 1 g of NaH_2PO_4 , 1.625 g of Na_2HPO_4 , and H_2O up to 250 mL) to avoid tissue degradation. The dehydrating and tissue clarification steps were performed using consecutive incubations with ethanol of increasing concentrations (70°, 90°, and 100°) and then with toluene (100°).

The samples were embedded in paraffin wax at 60 °C and sectioned with a microtome (Rotary Microtome model 45, Lipshaw, Detroit, Michigan, USA) with slide thickness set at 6 μ m. These sections were put on slides and kept at room temperature. Then, the slides were deparaffinized using toluene and washed with ethanol of decreasing concentrations (100°, 90°, and 70°) and water. Then, the staining with hematoxylin and eosin was performed, followed by dehydration in ethanol, clarification with toluene, and mounting in Canada balsam [34]. For each specimen, five slides were visualized on an Olympus BX43 light microscope (Olympus, Tokyo, Japan). The histological changes within the liver tissues were analyzed by randomly selected sections obtained for each fish as qualitative observation.

2.4. Statistical Analysis

Results were analyzed using the Student t-test (Microsoft Excel). Values were calculated as mean $\pm$ standard deviation (SD) expressed relative to control. The results were considered significant only if the *p* value was less than 0.05.

3. Results

3.1. Enzymatic Activity

CAT activity in the hepatic tissue from the 500 mg PVC group was not significantly lower than the levels from the control group. However, a drastic decrease of CAT activity was observed in the hepatic tissue of fish from the 1000 mg PVC group (Figure 1), suggesting a high accumulation of hydrogen peroxide and enzyme inhibition.

Glutathione (GSH) level, the most abundant cellular thiol antioxidant, was evaluated through the activity of the GPx enzyme, which exhibited significantly lower activities in the PVC-exposed groups compared to the control group (Figure 2).

Lipid peroxidation induced by PVC MPs was evaluated through the MDA values (Figure 3). The increasing MDA levels correlated with the CAT and GPx activity and indicated a rate of lipid peroxidation that increased with the concentration of the pollutant ($p < 0.001$ for 1000 mg PVC group compared to the control group).

3.2. Histological Observations

The liver of control fish exhibited normal architecture with uninucleate polygonal hepatocytes, embedded between blood sinusoids. The vesicular nucleus, provided with

a centrally placed nucleolus, had homogeneous chromatin. Most of the cells contained unstained cytoplasm (Figure 4A) or vacuolated, with eccentrically placed nuclei (Figure 4B). Cytoplasm vacuolization and nuclear hypertrophy (Figure 5A,B) were present in the 500 mg PVC-exposed group. Also, there were observed increased hepatocyte cytoplasm basophilia, cytoplasm vacuolation, nucleus, vacuolation, clumps of nuclear chromatin, necrosis, and leucocytes penetrating the walls of blood vessels and infiltrating the surrounding tissue (Figure 6A–D).

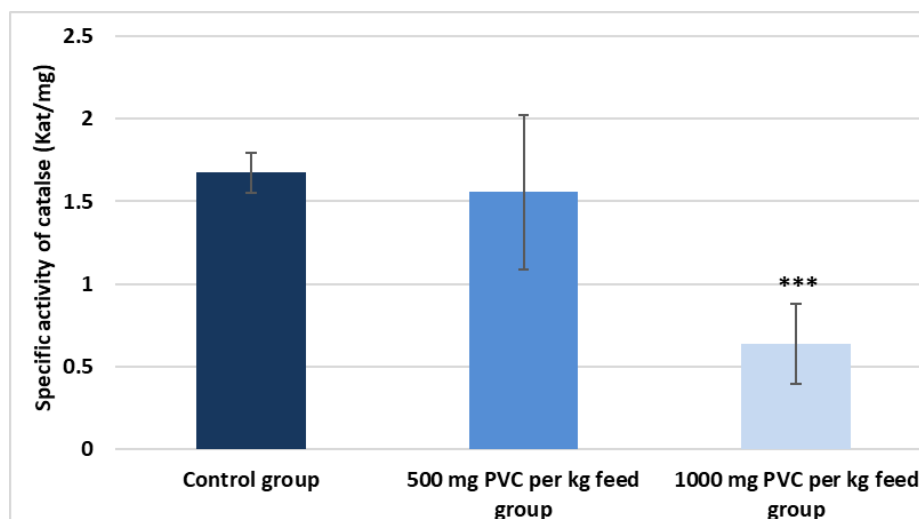


Figure 1. Catalase activity measured in the hepatic tissue from control and PVC-exposed groups. The results were expressed as mean values $\pm$ standard deviation ($n = 5$ specimens per group). *** $p < 0.001$ compared to control group.

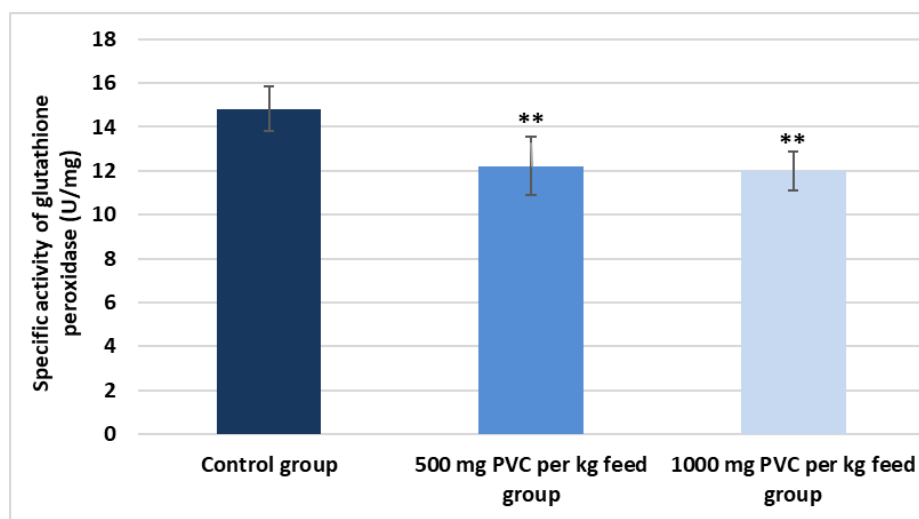


Figure 2. Glutathione peroxidase activity measured in the hepatic tissue from control and PVC-exposed groups. The results were expressed as mean values $\pm$ standard deviation ($n = 5$ specimens per group). ** $p < 0.01$ compared to control group.

In the 1000 mg PVC-exposed group, dilation of sinusoidal capillaries and mast cells was present (Figure 7), along with intercellular corridors and hyperchromatic nuclei (Figure 8) being observed. The vacuolated cytoplasm of the hepatocytes showed an increased eosinophilia, the nuclei underwent changes such as hypertrophy, pyknosis or karyolysis. Atypical lymphocytes and red blood cells appear in widely dilated sinusoidal capillaries; necrotic areas were observed as well (Figures 9–12).

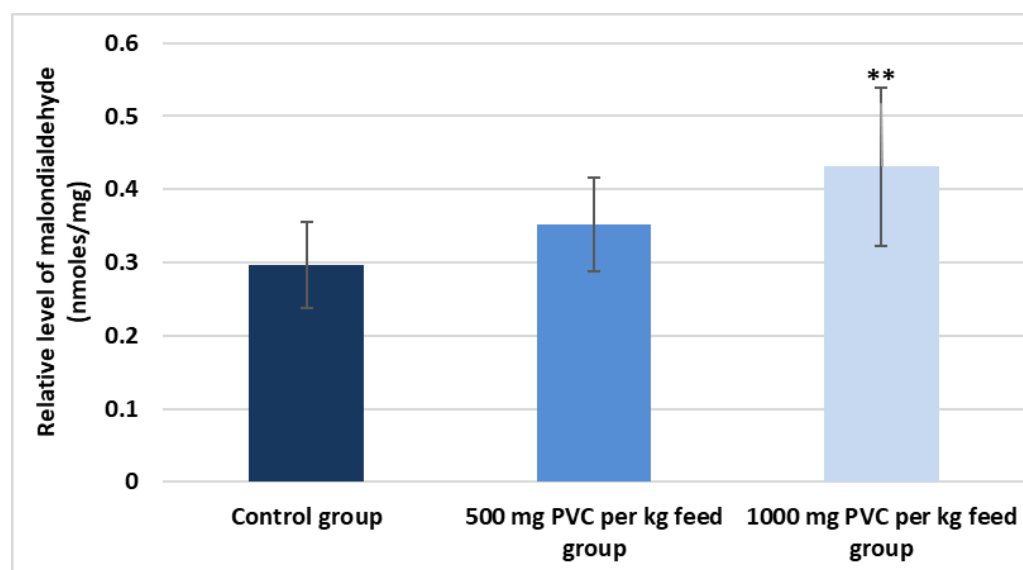


Figure 3. Malondialdehyde levels measured in the hepatic tissue of control and PVC-exposed groups. The results were expressed as mean values $\pm$ standard deviation ($n = 5$ specimens per group). ** $p < 0.01$ compared to control group.

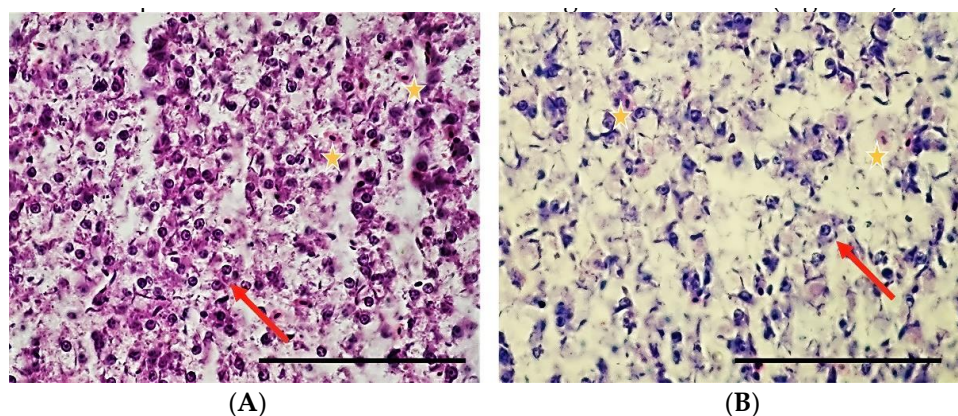


Figure 4. Microphotographs of hematoxylin–eosin staining of *Oreochromis niloticus* control liver revealed hepatocytes (arrows) and erythrocytes (stars) in sinusoid capillaries. Scale bar is 100 μm for both images.

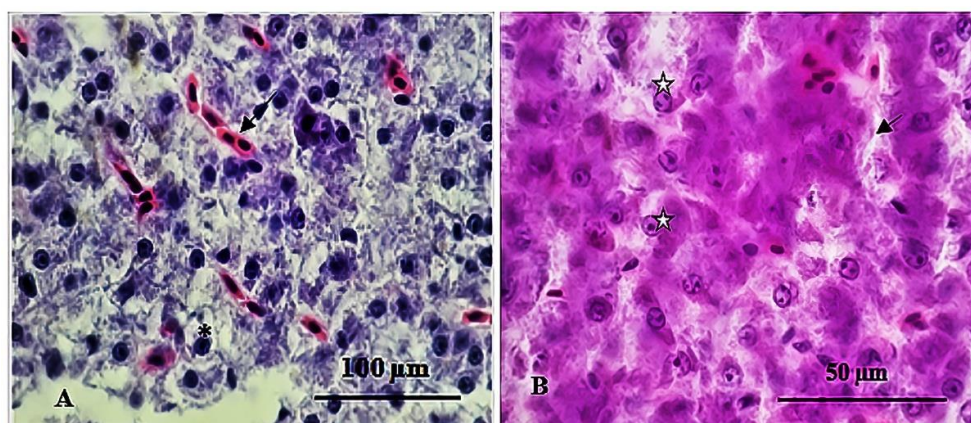


Figure 5. Microphotographs of hematoxylin–eosin staining of *Oreochromis niloticus* liver exposed to 500 mg PVC per kg feed revealed the following changes: (A) cytoplasm vacuolization (star), and (B) nuclear hypertrophy (white star) and formation of intercellular corridors (arrow).

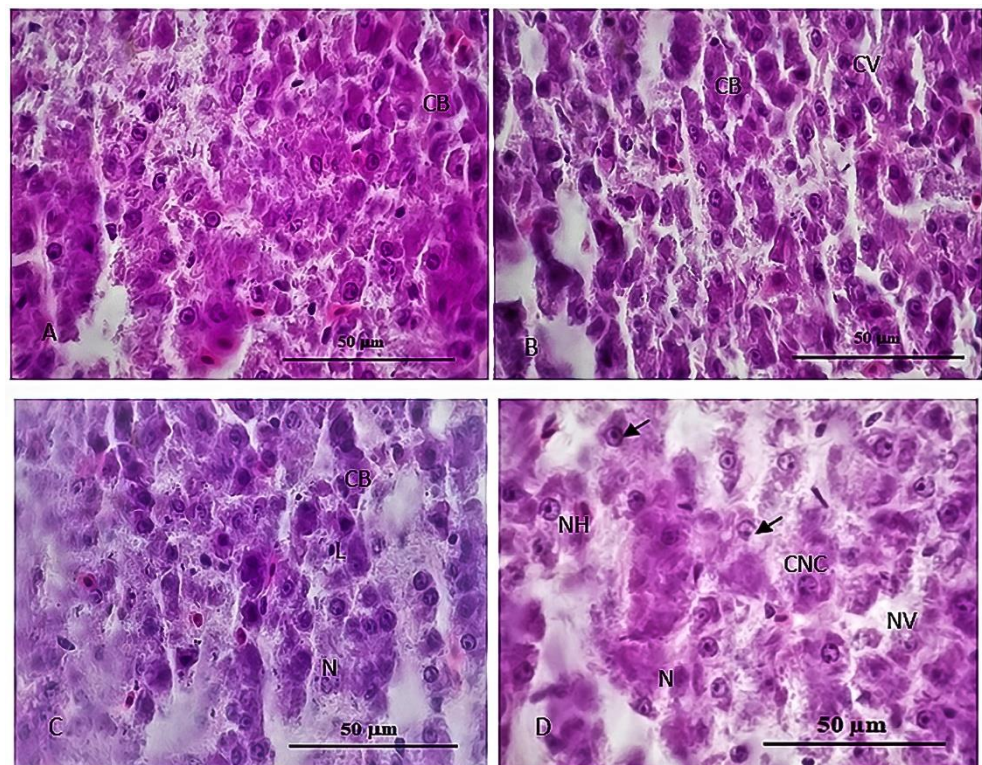


Figure 6. Microphotographs of *Oreochromis niloticus* liver, exposed to 500 mg PVC, hematoxylin-eosin stained to reveal regressive changes in liver tissue: alteration that affects the tissue structure (A–D), the cytoplasm (increased hepatocyte cytoplasm basophily (CB), cytoplasm vacuolation (CV), leucocytes penetrating the walls of blood vessels and infiltrating the surrounding tissue (L), nucleus hypertrophy (arrow), vacuolation (NV), the appearance of clumps of nuclear chromatin (CNC), and necrosis (N).

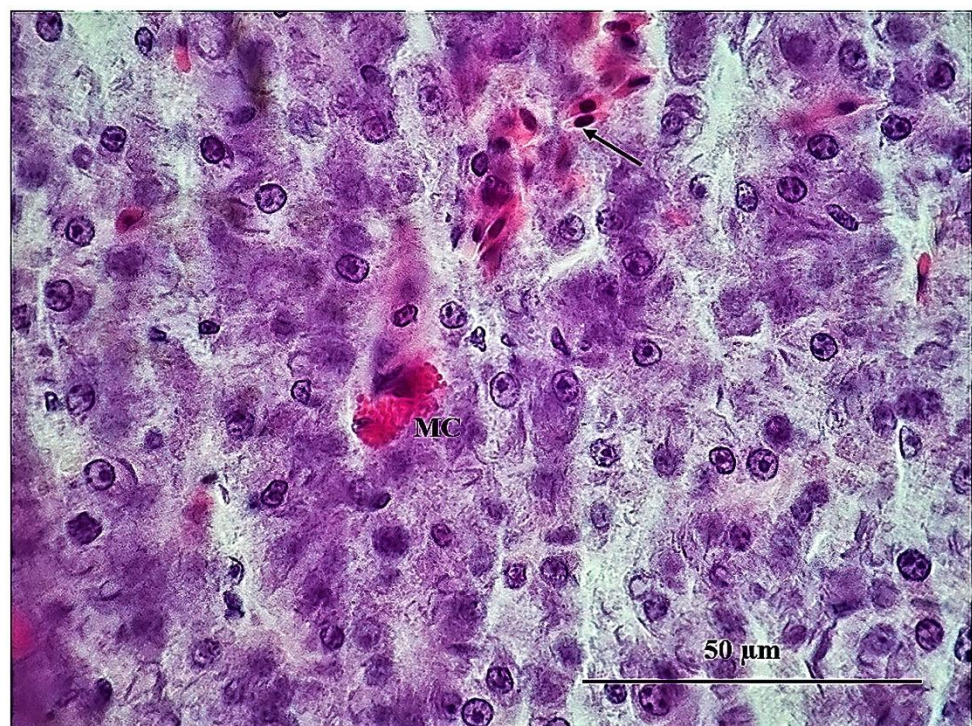


Figure 7. Microphotographs of *Oreochromis niloticus* liver, exposed to 1000 mg PVC (hematoxylin-eosin staining). Dilatation of sinusoidal capillaries (arrow), appearance of mast cells (MC).

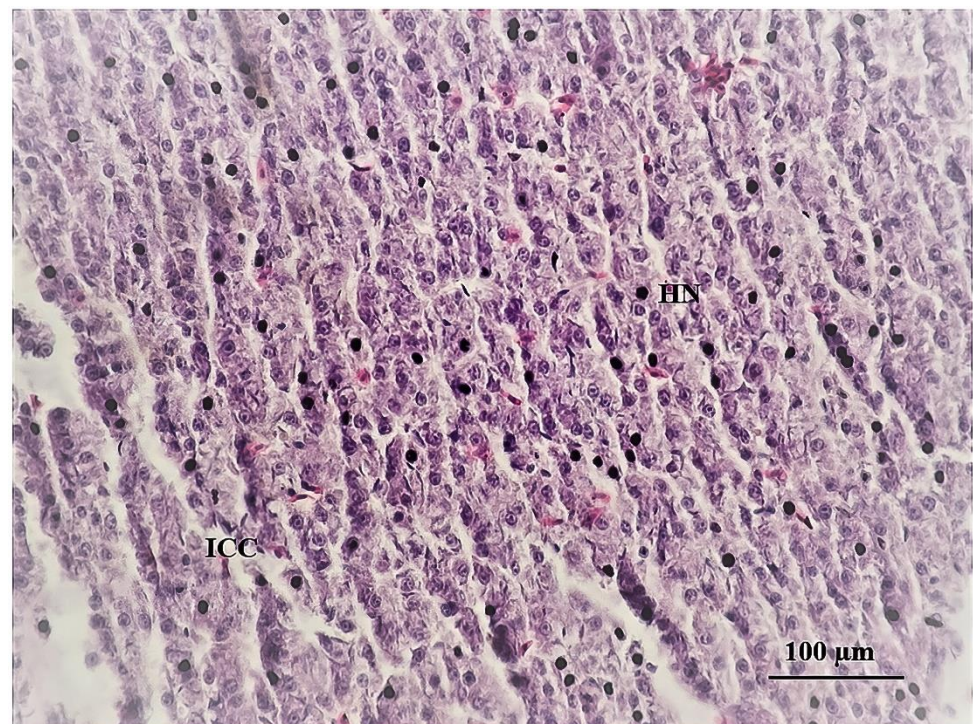


Figure 8. Microphotographs of *Oreochromis niloticus* liver, exposed to 1000 mg PVC (hematoxylin–eosin staining). The frequent occurrence of intercellular corridors (ICC) and hyperchromatic nuclei (HN) is noted.

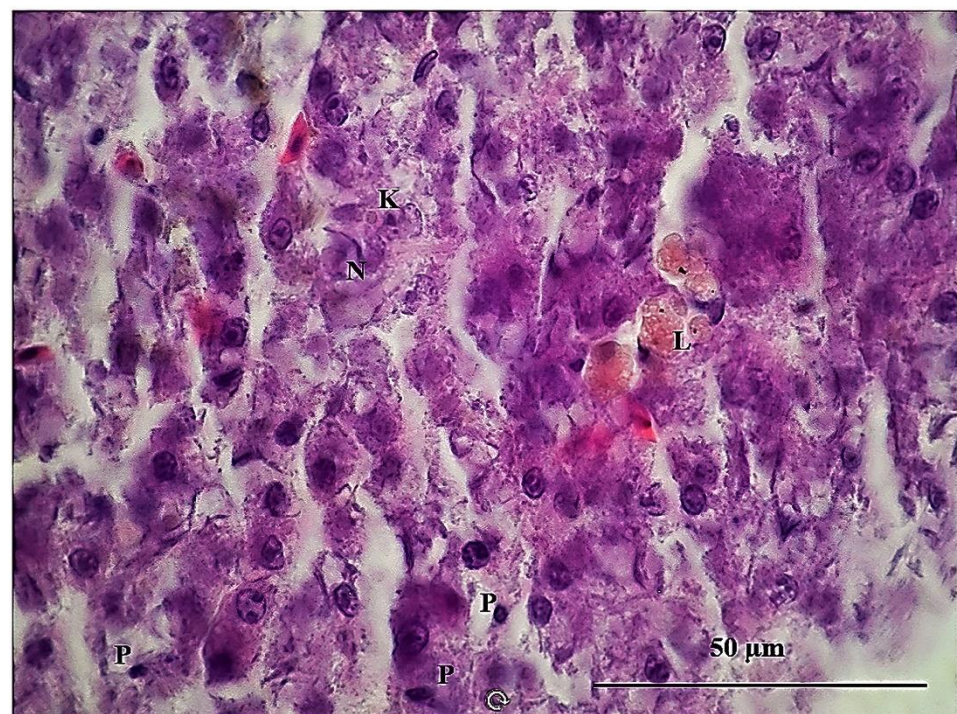


Figure 9. Microphotographs of *Oreochromis niloticus* liver, exposed to 1000 mg PVC (hematoxylin–eosin staining). The vacuolated cytoplasm of the hepatocytes shows an increased eosinophilia; the nuclei undergo changes such as hypertrophy, pyknosis (P) or karyolysis (K). Atypical lymphocytes (L) and red blood cells appear in widely dilated sinusoidal capillaries. Necrotic areas are observed (N).

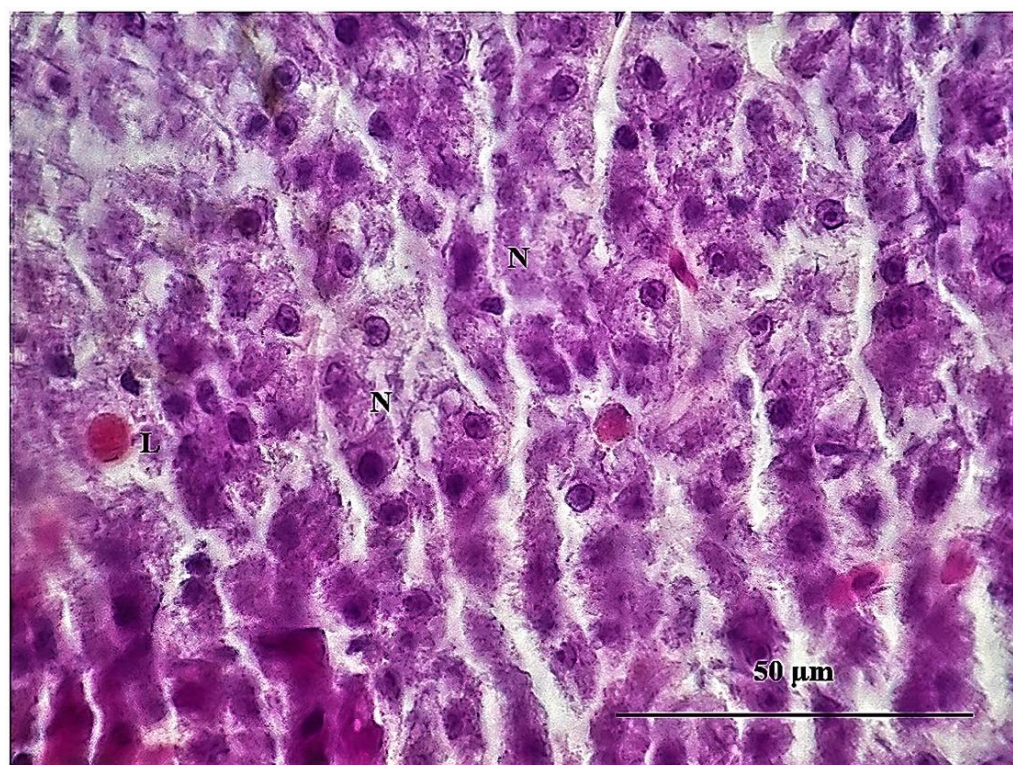


Figure 10. Microphotographs of *Oreochromis niloticus* liver, exposed to 1000 mg PVC (hematoxylin–eosin staining). Lymphocytes (L) and necrotic areas are observed (N).

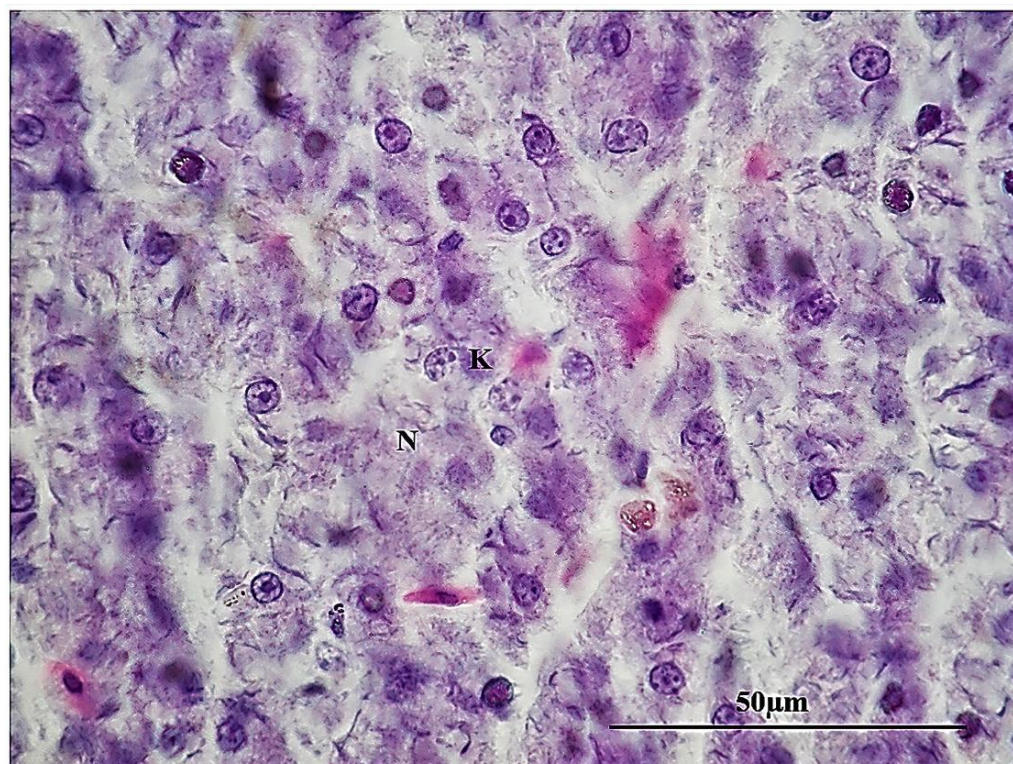


Figure 11. Microphotographs of *Oreochromis niloticus* liver, exposed to 1000 mg PVC (hematoxylin–eosin staining). Karyolysis (K) is seen in a necrotic region (N).

Intranuclear eosinophilic granules (Figure 12) and intranuclear granular material (Figure 13), as well as karyorrhexis, a nuclear modification specific to necrosis (Figure 14),

were also highlighted. The intensification of eosinophilia and hyalinization of the cytoplasm was evident in the necrotic region of the tissue (Figure 15).

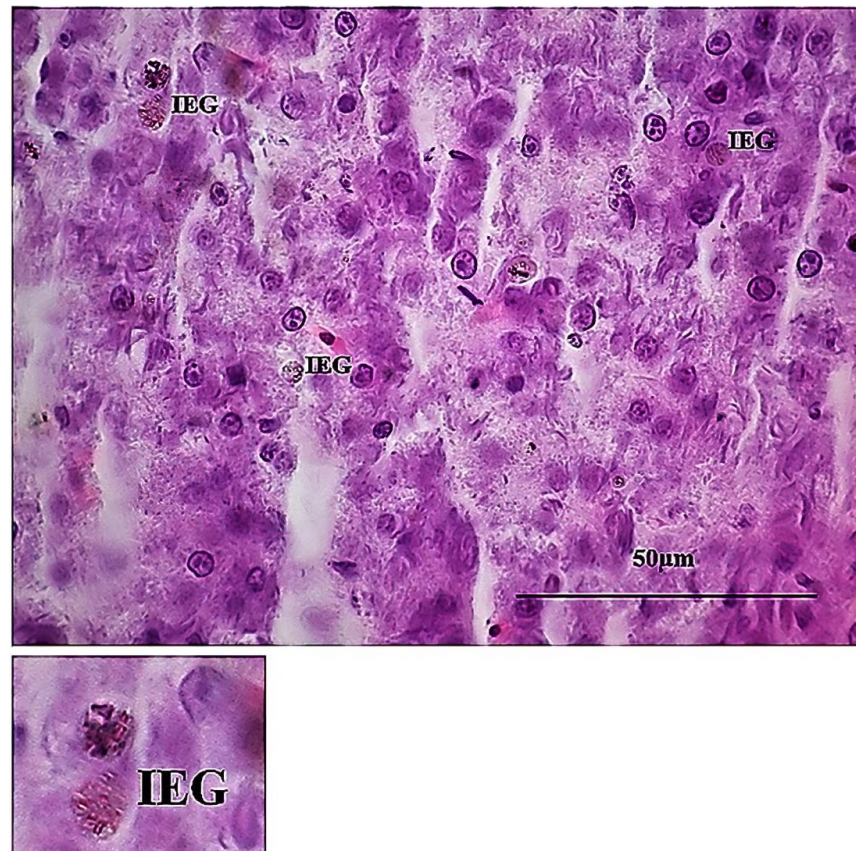


Figure 12. Microphotographs of *Oreochromis niloticus* liver, exposed to 1000 mg PVC (hematoxylin–eosin staining). Intranuclear eosinophilic granules (IEG) appear in the necrotic areas of the tissue.

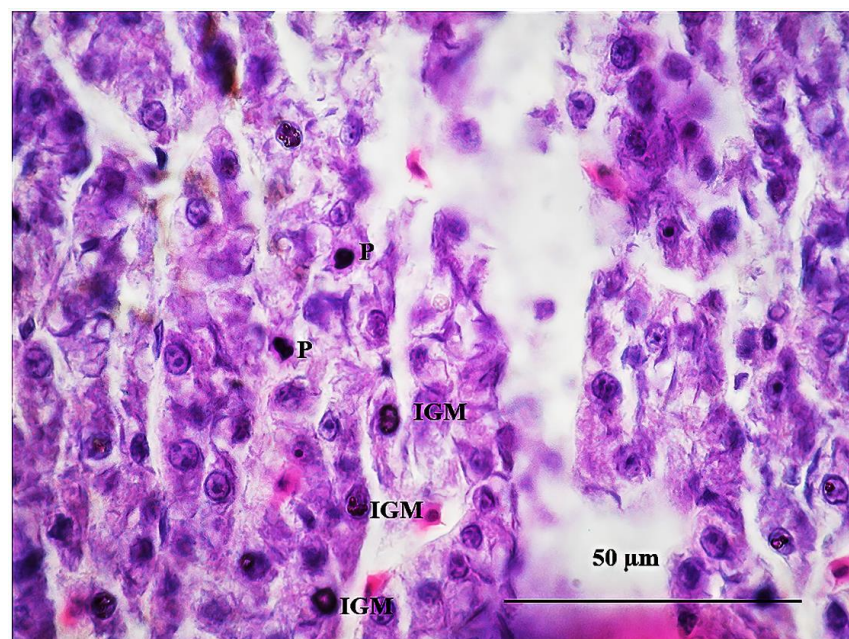


Figure 13. Microphotographs of *Oreochromis niloticus* liver, exposed to 1000 mg PVC (hematoxylin–eosin staining). Hepatocytes with pyknotic nuclei (P) and intranuclear granular material (IGM) are seen.

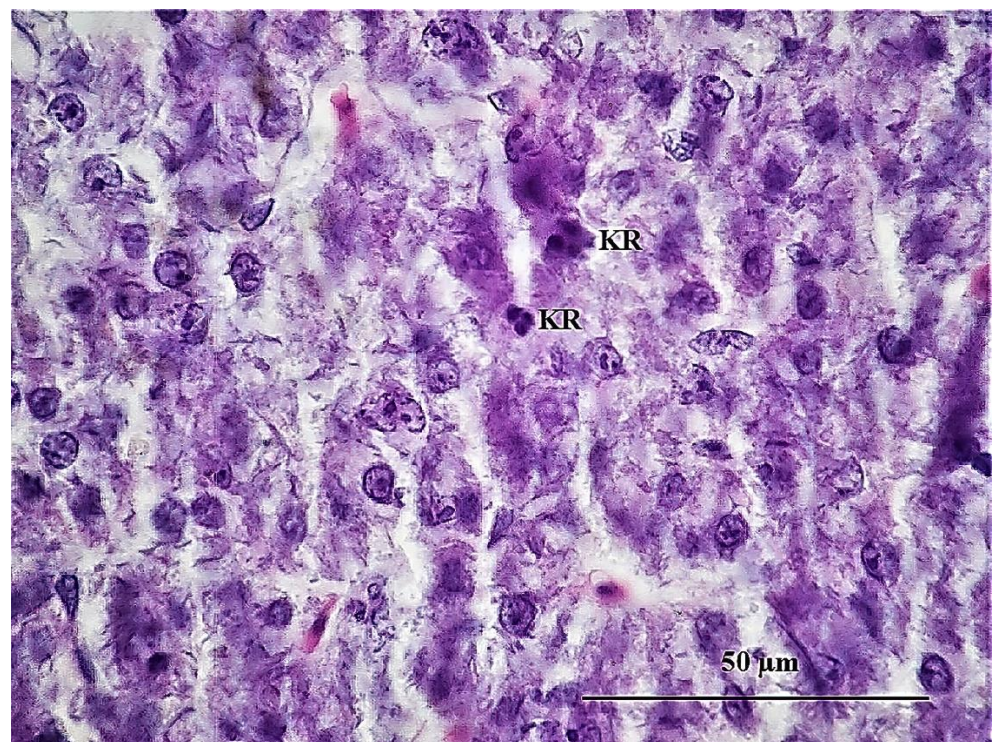


Figure 14. Microphotographs of *Oreochromis niloticus* liver, exposed to 1000 mg PVC (hematoxylin–eosin staining). In a necrotic area of the tissue, karyorrhexis is highlighted, a nuclear modification specific to this process, which consists in the fragmentation of the nuclei (KR).



Figure 15. Microphotographs of *Oreochromis niloticus* liver, exposed to 1000 mg PVC (hematoxylin–eosin staining). In a necrotic region of the tissue, the intensification of eosinophilia and hyalinization of the cytoplasm is evident.

4. Discussion

Liver histological observations provide robust indications of fish toxicity because this organ is primarily responsible for the detoxification and inactivation of exogenous compounds [32,35–37]. The fish liver features similar general circulatory components as the mammalian liver, blood being supplied by hepatic arterioles and portal veins, and drained by hepatic veins [37]. In many species belonging to Teleostei, such as Cichlidae (bony fish), the liver is divided into three lobes, however, no lobulation was recognized in some Teleostei [38]. *Oreochromis niloticus*, a bony fish, presents two hepatic lobes with hepatocytes being spread out as anastomotic cords, arranged in two cellular layers and surrounded by sinusoids [39]. In all fish species, the liver presents much of the same metabolic functions as those of mammals, including the metabolism of xenobiotic compounds. However, this metabolic function has a lower capacity than that found in mammals due to the ability of excretion of some toxic substances via gills. Furthermore, analogous mechanisms for managing xenobiotic compounds, including both phase I and phase II biotransformation reactions, and many of the same microsomal and cytosolic enzymes found in mammals, are also present in fish [37].

The goal of biotransformation is to produce metabolites that are more hydrophilic than the parent compound, and therefore, more easily excreted. Due to certain anatomic and physiologic considerations, hepatic toxicity in fish tends to be less severe than in mammals, and fish do not generally display a zonal response pattern of hepatic intoxication. Morphologic features of liver toxicity are often exacerbations of findings that may be observed in control fish [37]. Therefore, observations of such modifications need to be correlated with other markers, such as oxidative stress.

Oxidative stress is quantified by the balance between the activity of antioxidant enzymes and the level of lipid peroxidation, as well as direct quantification of reactive oxygen species (ROS). The alteration of antioxidant enzymes activity and the elevation of MDA levels which indicate lipid peroxidation in fish exposed to different pollutants, can be regarded as bioindicators of oxidative stress [40–43]. Studies have identified different oxidative stress responses which are triggered through exposure to microplastics, mainly by dysregulations of CAT and superoxide dismutase enzymes, as well as higher MDA levels, suggesting a strong link between MPs pollution and liver enzymatic imbalance in fish [43,44]. It was also reported that the size of PVC MPs showed a significant role in fish toxicity and mortality, as PVC particles in the size range of $\approx 100\ \mu\text{m}$ were more toxic than those in the size range of $100\text{--}1000\ \mu\text{m}$ [45]. In our study, the exposure to $1000\ \text{mg PVC per kg of feed}$ decreased the activity of CAT compared to the control group in a significant way (Figure 1), confirming the perturbation in the antioxidative homeostasis of *Oreochromis niloticus* liver. Although there were no important differences between PVC-exposed groups in terms of GPx activity (Figure 2), a dose-dependent increase was noticed for MDA level (Figure 3). These changes could be explained by a high concentration of ROS (such as hydrogen peroxide and hydroperoxides) that alter the normal activities of CAT and GPx, with negative consequences on lipid peroxidation that affected the hepatocytes' structure. Previously, the same pattern of oxidative stress and hepatic damage induced by PVC was also revealed in *Clarias gariepinus* and *O. niloticus* as well [43,46]. However, as no other study is currently available on this size of PVC MPs and *O. niloticus*, as far as we know, and considering the toxicological impacts of PVC MPs being shown on other fish species such as on muscle tissue of *Etroplus suratensis* [47] and liver of *Sparus aurata* L. [48], our findings on *O. niloticus* are of significant importance for environmental science regarding microplastic pollution. Considering that other reports confirmed a greater resistance of *O. niloticus* to various xenobiotics [46,47], we can state that PVC exposure in the feed can significantly alter fish life and consequently ecosystem health, including human health, as MPs can migrate into the muscle tissue [2,43].

Although still trailing behind in terms of available data, the genotoxic effects of microplastics in fish are now beginning to be explored alongside other classic ecotoxicological effects (oxidative stress, reproductive impairment, feeding dysfunctions, etc.) [49].

In fish, like in other organisms, ROS causes DNA damage through a variety of processes, such as base and/or sugar alterations, sugar–base cyclization, DNA–protein crosslinks, and intra- and interstrand crosslinks [50]. The genotoxicity of microplastics could be the result of direct interaction with DNA molecules or indirect mechanisms by free radicals' overproduction and oxidative stress [51], DNA damage being responsible for histopathologic changes in liver tissue [52,53]. Antamanalp et al. reported that MPs exposure induces lipid peroxidation in a concentration-dependent manner [43], and although ROS generation is now well-correlated with MP pollution, very few reports, if any, specify the immunological aspects triggered by such exposure in *O. niloticus*.

Morphological studies of fish immune systems can be considered somewhat forgotten or ignored, an example being the lack of acknowledgment of a lymphatic vessel system in fish [54]. In addition, another fact not well acknowledged is that fish may have lymphatic vessels related to the intestines [55], as an elaborate system of lymphatic vessels. Such was found present in the common wolffish intestine, with Mast cells (MCs) being closely associated with lymphatic vessels, however, the same was not identified in rainbow trout [55]. The fish MCs, also known as eosinophilic granule cell (EGCs) [56], originate from hematopoietic organs, and although fish lack bone marrow and lymph nodes, they have a lymphatic system that is mainly composed of the head, kidney, spleen, thymus, and mucosa-associated lymphoid tissue. The digestive tract is usually the richest source of MCs/EGCs in fish, especially in parasitic infestation [57], as teleost MCs/EGCs show close functional similarity to the mast cells of mammals, being motile and able to migrate to the site of infection and/or tissue damage where they release mediators of inflammation [58]. Although there are no identical distribution patterns or morphological features that mast cells exhibit between fish species [59], the tissue distribution of MCs/EGCs in species from a certain genus shows a characteristic pattern, which is commonly also present at the family level [58]. In terms of plastic exposure, there are studies indicating that plastic ingestion in fish induces hepatic [60], as well as innate immune system [61] stress.

5. Conclusions

The present study indicates that a midterm exposure to PVC MPs that are at the lower end of MPs size spectrum ($\approx 50 \mu\text{m}$ Ø) can induce toxic effects in *Oreochromis niloticus* via elevated oxidative stress levels. Histopathological changes also occurred in the exposed groups and the oxidative stress was initiated in a dose-dependent manner, with consistent differences between the exposed groups and the control group, as reported by other authors that evaluated MPs of various other sizes [23,30,35,46]. In conclusion, these findings contribute to a better understanding of the toxicity of PVC MPs ingestion in *O. niloticus*, highlighting the need for a MPs size-related hazards classification. Furthermore, a deeper investigation into cell signaling pathways that are modulating the PVC toxicity, especially those involved in the generated immune response, should be continued in further studies.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/su15086494/s1>, Table S1. Water Parameters during the 40 days study period.

Author Contributions: Conceptualization, A.J., N.C. and D.F.M.; methodology, M.S.S., C.A.S., A.J. and R.-I.V.; software, M.S.S.; validation, D.F.M., C.A.S. and G.D.; formal analysis, D.F.M.; investigation, N.C.; resources, C.A.S. and N.C.; data curation, C.A.S., G.D. and D.F.M.; writing—original draft preparation, A.J., M.-V.B. and R.-I.V.; writing—review and editing, A.J., C.A.S. and D.F.M.; visualization, G.D.; supervision, D.F.M., C.A.S. and G.D.; project administration, G.D. All authors have read and agreed to the published version of the manuscript.

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References

1. Iroegbu, A.O.C.; Ray, S.S.; Mbarane, V.; Bordado, J.C.; Sardinha, J.P. Plastic Pollution: A Perspective on Matters Arising: Challenges and Opportunities. *ACS Omega* **2021**, *6*, 19343–19355. [CrossRef] [PubMed]
2. Farady, S.E. Microplastics as a new, ubiquitous pollutant: Strategies to anticipate management and advise seafood consumers. *Mar. Policy* **2019**, *104*, 103–107. [CrossRef]
3. Schwarz, A.E.; Lighthart, T.N.; Boukris, E.; van Harmelen, T. Sources, transport, and accumulation of different types of plastic litter in aquatic environments: A review study. *Mar. Pollut. Bull.* **2019**, *143*, 92–100. [CrossRef] [PubMed]
4. Jeong, C.-B.; Won, E.-J.; Kang, H.-M.; Lee, M.-C.; Hwang, D.-S.; Hwang, U.-K.; Zhou, B.; Souissi, S.; Lee, S.-J.; Lee, J.-S. Microplastic Size-Dependent Toxicity, Oxidative Stress Induction, and p-JNK and p-p38 Activation in the Monogonont Rotifer (*Brachionus koreanus*). *Environ. Sci. Technol.* **2016**, *50*, 8849–8857. [CrossRef]
5. Wilhelm, R. Resin identification codes—New astm standard based on society of the plastics industry code will facilitate recycling. In *Standardization News*; ASTM International: West Conshohocken, PA, USA, 2016.
6. Andrady, A.L.; Neal, M.A. Applications and societal benefits of plastics. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2009**, *364*, 1977–1984. [CrossRef]
7. Polyvinyl Chloride—Chemical Safety Facts. 2023. Available online: <https://www.chemicalsafetyfacts.org/chemicals/polyvinyl-chloride/> (accessed on 10 February 2022).
8. Fanelli, R.; Zuccato, E. Risks and benefits of PVC in medical applications. *Boll. Chim. Farm.* **2002**, *141*, 282–289. [PubMed]
9. Esterhuizen, M.; Kim, Y.J. Effects of polypropylene, polyvinyl chloride, polyethylene terephthalate, polyurethane, high-density polyethylene, and polystyrene microplastic on *Nelumbo nucifera* (Lotus) in water and sediment. *Environ. Sci. Pollut. Res. Int.* **2022**, *29*, 17580–17590. [CrossRef]
10. Wu, P.; Tang, Y.; Jin, H.; Song, Y.; Liu, Y.; Cai, Z. Consequential fate of bisphenol-attached PVC microplastics in water and simulated intestinal fluids. *Environ. Sci. Ecotechnol.* **2020**, *2*, 100027. [CrossRef]
11. Silva, A.C.P.; Jorgetto, A.O.; Wondracek, M.H.P.; Saeki, M.J.; Pedrosa, V.A.; Colmenares, Y.N.; Mastelaro, V.R.; Sutili, F.K.; Martinez, M.A.U.; Pasta, P.C.; et al. A global pollutant (PVC-polyvinyl chloride) applied as heavy metal binder from aqueous samples: Green principles from synthesis to application. *Environ. Technol.* **2021**, *43*, 3742–3754. [CrossRef]
12. Thompson, R.C.; Moore, C.J.; vom Saal, F.S.; Swan, S.H. Plastics, the environment and human health: Current consensus and future trends. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2009**, *364*, 2153–2166. [CrossRef]
13. Schwarzer, M.; Brehm, J.; Vollmer, M.; Jasinski, J.; Xu, C.; Zainuddin, S.; Fröhlich, T.; Schott, M.; Greiner, A.; Scheibel, T.; et al. Shape, size, and polymer dependent effects of microplastics on *Daphnia magna*. *J. Hazard. Mater.* **2022**, *426*, 128136. [CrossRef] [PubMed]
14. Gray, A.D.; Weinstein, J.E. Size- and shape-dependent effects of microplastic particles on adult daggerblade grass shrimp (*Palaemonetes pugio*). *Environ. Toxicol. Chem.* **2017**, *36*, 3074–3080. [CrossRef] [PubMed]
15. Issac, M.N.; Kandasubramanian, B. Effect of microplastics in water and aquatic systems. *Environ. Sci. Pollut. Res. Int.* **2021**, *28*, 19544–19562. [CrossRef]
16. Gesamp. Sources, Fate and Effects of Microplastics in the Marine Environment. Available online: <http://www.gesamp.org/publications/reports-and-studies-no-90> (accessed on 15 February 2022).
17. Arthur, C.; Baker, J.E.; Bamford, H.A. *Proceedings of the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris*, Tacoma, WA, USA, 9–11 September 2008; University of Washington Tacoma: Tacoma, WA, USA, 2008.
18. Hartmann, N.B.; Hüffer, T.; Thompson, R.C.; Hassellöv, M.; Verschoor, A.; Dagaard, A.E.; Rist, S.; Karlsson, T.; Brennholt, N.; Cole, M.; et al. Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris. *Environ. Sci. Technol.* **2019**, *53*, 1039–1047. [CrossRef] [PubMed]
19. Jåms, I.B.; Windsor, F.M.; Poudevigne-Durance, T.; Ormerod, S.J.; Durance, I. Estimating the size distribution of plastics ingested by animals. *Nat. Commun.* **2020**, *11*, 1594. [CrossRef]
20. Jamieson, A.J.; Brooks, L.S.R.; Reid, W.D.K.; Pierny, S.B.; Narayanaswamy, B.E.; Linley, T.D. Microplastics and synthetic particles ingested by deep-sea amphipods in six of the deepest marine ecosystems on Earth. *R. Soc. Open Sci.* **2019**, *6*, 180667. [CrossRef]
21. Zantis, L.J.; Carroll, E.L.; Nelms, S.E.; Bosker, T. Marine mammals and microplastics: A systematic review and call for standardisation. *Environ. Pollut.* **2021**, *269*, 116142. [CrossRef]
22. A Plague of Plastics. Available online: <https://www.nwf.org/Home/Magazines/National-Wildlife/2019/June-July/Conservation/Ocean-Plastic> (accessed on 10 March 2022).
23. Zhang, C.; Zuo, Z.; Wang, Q.; Wang, S.; Lv, L.; Zou, J. Size Effects of Microplastics on Embryos and Observation of Toxicity Kinetics in Larvae of Grass Carp (*Ctenopharyngodon idella*). *Toxics* **2022**, *10*, 76. [CrossRef]

24. Gigault, J.; Halle, A.t.; Baudrimont, M.; Pascal, P.-Y.; Gauffre, F.; Phi, T.-L.; El Hadri, H.; Grassl, B.; Reynaud, S. Current opinion: What is a nanoplastic? *Environ. Pollut.* **2018**, *235*, 1030–1034. [\[CrossRef\]](#)
25. Molenaar, R.; Chatterjee, S.; Kamphuis, B.; Segers-Nolten, I.M.J.; Claessens, M.M.A.E.; Blum, C. Nanoplastic sizes and numbers: Quantification by single particle tracking. *Environ. Sci. Nano* **2021**, *8*, 723–730. [\[CrossRef\]](#)
26. Hollerová, A.; Hodkovicová, N.; Blahová, J.; Faldyna, M.; Maršálek, P.; Svobodová, Z. Microplastics as a potential risk for aquatic environment organisms—A review. *Acta Vet. Brno* **2021**, *90*, 99–107. [\[CrossRef\]](#)
27. Rairat, T.; Liu, Y.-K.; Hsu, J.C.-N.; Hsieh, C.-Y.; Chuchird, N.; Chou, C.-C. Combined Effects of Temperature and Salinity on the Pharmacokinetics of Florfenicol in Nile Tilapia (*Oreochromis niloticus*) Reared in Brackish Water. *Front. Vet. Sci.* **2022**, *9*, 826586. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Figueiredo-Fernandes, A.; Fontainhas-Fernandes, A.; Peixoto, F.; Rocha, E.; Reis-Henriques, M.A. Effects of gender and temperature on oxidative stress enzymes in Nile tilapia *Oreochromis niloticus* exposed to paraquat. *Pestic. Biochem. Physiol.* **2006**, *85*, 97–103. [\[CrossRef\]](#)
29. Aryani, D.; Khalifa, M.A.; Herjayanto, M.; Solahudin, E.A.; Rizki, E.M.; Halwatiyah, W.; Istiqomah, H.; Maharani, S.H.; Wahyudin, H.; Pratama, G. Penetration of Microplastics (Polyethylene) to Several Organs of Nile Tilapia (*Oreochromis niloticus*). In *IOP Conference Series: Earth and Environmental Science*; IOP Publishing: Bristol, UK, 2021; Volume 715, p. 012061. [\[CrossRef\]](#)
30. Hamed, M.; Soliman, H.A.M.; Osman, A.G.M.; Sayed, A.E.-D.H. Antioxidants and molecular damage in Nile Tilapia (*Oreochromis niloticus*) after exposure to microplastics. *Environ. Sci. Pollut. Res. Int.* **2020**, *27*, 14581–14588. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Hamed, M.; Soliman, H.A.M.; Osman, A.G.M.; Sayed, A.E.-D.H. Assessment the effect of exposure to microplastics in Nile Tilapia (*Oreochromis niloticus*) early juvenile: I. blood biomarkers. *Chemosphere* **2019**, *228*, 345–350. [\[CrossRef\]](#)
32. Ding, J.; Zhang, S.; Razanajatovo, R.M.; Zou, H.; Zhu, W. Accumulation, tissue distribution, and biochemical effects of polystyrene microplastics in the freshwater fish red tilapia (*Oreochromis niloticus*). *Environ. Pollut.* **2018**, *238*, 1–9. [\[CrossRef\]](#)
33. Close, B.; Banister, K.; Baumans, V.; Bernoth, E.-M.; Bromage, N.; Bunyan, J.; Erhardt, W.; Flecknell, P.; Gregory, N.; Hackbarth, H.; et al. Recommendations for euthanasia of experimental animals: Part 2. *Lab. Anim.* **1997**, *31*, 1–32. [\[CrossRef\]](#)
34. Mumford, S.H.J.; Smith, C.; Morrison, J.; Macconnell, B.; Blazer, V. *Fish Histology and Histopathology*; US Fish & Wildlife Service: Elkins, WV, USA, 2007; Volume 4.
35. Alomar, C.; Sureda, A.; Capó, X.; Guijarro, B.; Tejada, S.; Deudero, S. Microplastic ingestion by *Mullus surmuletus* Linnaeus, 1758 fish and its potential for causing oxidative stress. *Environ. Res.* **2017**, *159*, 135–142. [\[CrossRef\]](#)
36. Wang, M.; Xu, G.; Tang, Y.; Su, S.; Wang, Y.; Zhu, Z. Investigation of the Molecular Mechanisms of Antioxidant Damage and Immune Response Downregulation in Liver of *Coilia nasus* Under Starvation Stress. *Front. Endocrinol.* **2021**, *12*, 622315. [\[CrossRef\]](#)
37. Wolf, J.C.; Wolfe, M.J. A Brief Overview of Nonneoplastic Hepatic Toxicity in Fish. *Toxicol. Pathol.* **2005**, *33*, 75–85. [\[CrossRef\]](#)
38. Bruslé, J.; González i Anadon, G. The Structure and Function of Fish Liver. *Fish Morphol.* **2017**, *1*, 77–93. [\[CrossRef\]](#)
39. Vicentini, C.A.; Franceschini-Vicentini, I.B.; Bombonato, M.T.S.; Bertolucci, B.; Lima, S.G.; Santos, A.S. Morphological Study of the Liver in the Teleost *Oreochromis niloticus*. *Int. J. Morphol.* **2005**, *23*, 211–216. [\[CrossRef\]](#)
40. Rahimikia, E. Analysis of antioxidants and serum biochemical responses in goldfish under nickel exposure by sub-chronic test. *J. Appl. Anim. Res.* **2016**, *45*, 320–325. [\[CrossRef\]](#)
41. Vinodhini, R.; Narayanan, M. Biochemical changes of antioxidant enzymes in common carp (*Cyprinus carpio* L.) after heavy metal exposure. *Turk. J. Vet. Anim. Sci.* **2009**, *33*, 273–278. [\[CrossRef\]](#)
42. Shah, Z.U.; Parveen, S. Oxidative, biochemical and histopathological alterations in fishes from pesticide contaminated river Ganga, India. *Sci. Rep.* **2022**, *12*, 3628. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Atamanalp, M.; Korkturk, M.; Kırıcı, M.; Ucar, A.; Kırıcı, M.; Parlak, V.; Aydın, A.; Alak, G. Interaction of Microplastic Presence and Oxidative Stress in Freshwater Fish: A Regional Scale Research, East Anatolia of Türkiye (Erzurum & Erzincan & Bingöl). *Sustainability* **2022**, *14*, 12009. [\[CrossRef\]](#)
44. Qiao, R.; Sheng, C.; Lu, Y.; Zhang, Y.; Ren, H.; Lemos, B. Microplastics induce intestinal inflammation, oxidative stress, and disorders of metabolome and microbiome in zebrafish. *Sci. Total Environ.* **2019**, *662*, 246–253. [\[CrossRef\]](#)
45. Darabi, H.; Baradaran, A.; Ebrahimpour, K. Subacute toxic effects of polyvinyl chloride microplastics (PVC-MPs) in juvenile common carp, *Cyprinus carpio* (Pisces: Cyprinidae). *Casp. J. Environ. Sci.* **2022**, *20*, 233–242. [\[CrossRef\]](#)
46. Iheanacho, S.C.; Odo, G.E. Dietary Exposure to Polyvinyl Chloride Microparticles Induced Oxidative Stress and Hepatic Damage polyvinyl chloride microparticles induced oxidative stress and hepatic damage in *Clarias Gariepinus gariepinus* (Burchell, 1822). *Int. Environ. Sci. Pollut. Res.* **2020**, *27*, 21159–21173. [\[CrossRef\]](#)
47. Vijayaraghavan, G.; Neethu, K.V.; Aneesh, B.P.; Suresh, A.; Saranya, K.S.; Bijoy Nandan, S.; Sharma, K.V. Evaluation of toxicological impacts of Polyvinyl Chloride (PVC) microplastics on fish, *Etroplus suratensis* (Bloch, 1790), Cochin estuary, India. *Toxicol. Environ. Health Sci.* **2022**, *14*, 131–140. [\[CrossRef\]](#)
48. Espinosa, C.; Cuesta, A.; Esteban, M.Á. Effects of dietary polyvinylchloride microparticles on general health, immune status and expression of several genes related to stress in gilthead seabream (*Sparus aurata* L.). *Fish Shellfish. Immunol.* **2017**, *68*, 251–259. [\[CrossRef\]](#) [\[PubMed\]](#)
49. Miloloža, M.; Kučić Grgić, D.; Bolanča, T.; Ukić, Š.; Cvetnić, M.; Ocelić Bulatović, V.; Dionysiou, D.D.; Kušić, H. Ecotoxicological Assessment of Microplastics in Freshwater Sources—A Review. *Water* **2020**, *13*, 56. [\[CrossRef\]](#)
50. Gonzalez-Hunt, C.P.; Wadhwa, M.; Sanders, L.H. DNA damage by oxidative stress: Measurement strategies for two genomes. *Curr. Opin. Toxicol.* **2018**, *7*, 87–94. [\[CrossRef\]](#)

51. Manke, A.; Wang, L.; Rojanasakul, Y. Mechanisms of nanoparticle-induced oxidative stress and toxicity. *Biomed. Res. Int.* **2013**, *2013*, 942916. [[CrossRef](#)] [[PubMed](#)]
52. Topal, A.; Gergit, A.; Özkaraca, M. Assessment of oxidative DNA damage, oxidative stress responses and histopathological alterations in gill and liver tissues of *Oncorhynchus mykiss* treated with linuron. *Hum. Exp. Toxicol.* **2020**, *40*, 1112–1121. [[CrossRef](#)]
53. Sun, J.-L.; Zhao, L.-L.; Liao, L.; Tang, X.-H.; Cui, C.; Liu, Q.; He, K.; Ma, J.-D.; Jin, L.; Yan, T.; et al. Interactive effect of thermal and hypoxia on largemouth bass (*Micropterus salmoides*) gill and liver: Aggravation of oxidative stress, inhibition of immunity and promotion of cell apoptosis. *Fish Shellfish. Immunol.* **2020**, *98*, 923–936. [[CrossRef](#)]
54. Bjørgen, H.; Koppang, E.O. Anatomy of teleost fish immune structures and organs. *Immunogenetics* **2021**, *73*, 53–63. [[CrossRef](#)]
55. Hellberg, H.; Bjerkås, I.; Vågnes, Ø.B.; Noga, E.J. Mast cells in common wolffish *Anarhichas lupus* L.: Ontogeny, distribution and association with lymphatic vessels. *Fish Shellfish. Immunol.* **2013**, *35*, 1769–1778. [[CrossRef](#)]
56. Roberts, R.J.; Young, H.; Milne, J.A. Studies on the skin of plaice (*Pleuronectes platessa* L.). *J. Fish Biol.* **1972**, *4*, 87–98. [[CrossRef](#)]
57. Sfacteria, A.; Brines, M.; Blank, U. The mast cell plays a central role in the immune system of teleost fish. *Mol. Immunol.* **2015**, *63*, 3–8. [[CrossRef](#)] [[PubMed](#)]
58. Reite, O.B.; Evensen, Ø. Inflammatory cells of teleostean fish: A review focusing on mast cells/eosinophilic granule cells and rodlet cells. *Fish Shellfish. Immunol.* **2006**, *20*, 192–208. [[CrossRef](#)] [[PubMed](#)]
59. Baccari, G.C.; Pinelli, C.; Santillo, A.; Minucci, S.; Rastogi, R.K. Mast Cells in Nonmammalian Vertebrates. *Int. Rev. Cell Mol. Biol.* **2011**, *290*, 1–53. [[CrossRef](#)] [[PubMed](#)]
60. Rochman, C.M.; Hoh, E.; Kurobe, T.; Teh, S.J. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. *Sci. Rep.* **2013**, *3*, 3263. [[CrossRef](#)] [[PubMed](#)]
61. Greven, A.-C.; Merk, T.; Karagöz, F.; Mohr, K.; Klapper, M.; Jovanović, B.; Palić, D. Polycarbonate and polystyrene nanoplastic particles act as stressors to the innate immune system of fathead minnow (*Pimephales promelas*). *Environ. Toxicol. Chem.* **2016**, *35*, 3093–3100. [[CrossRef](#)] [[PubMed](#)]

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