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Oncorhynchus mykiss Microplastic Exposure: Impacts and Research Gaps

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ABSTRACT
Worldwide, this review brings together scientific studies examining the effects of microplastics, a major pollutant in freshwater ecosystems, on rainbow trout (*Oncorhynchus mykiss*). Data such as microplastic types, concentrations, exposure times and biological responses of fish (histopathological changes in tissues, immune responses and biochemical parameters) were collected and evaluated from these studies. Microplastics accumulate in the biological systems of farmed trout in particular and cause toxic effects. Additionally, the effects of microplastics on protein, amino acid and fatty acid composition in fish are included in the dataset. The collected data were transferred to SPSS program and analyzed using descriptive statistics. The biological effects of microplastic and some pollutant combinations and experimental periods were evaluated by comparative analyses.As a result of the analysis, it was revealed that the combination of different types of microplastics with pesticides and heavy metals caused histopathological and biochemical changes in fish tissues. It was determined that organs such as gills and liver were more susceptible to microplastic exposure, especially at high concentrations. It has also been determined that some pollutant combinations with microplastic cause negative effects on oxidative stress, amino acid and fatty acid compositions. The shortcomings of existing research on the effects of microplastics on the biological systems of fish and the inability of laboratory experiments to explain the environmental distribution and biological effects of microplastics observed in natural environments are discussed. It was concluded that experiments conducted in laboratory environments do not adequately reflect natural environmental conditions and that more realistic assessments such as environmental risk assessments are needed. It is stated that these findings require comprehensive studies to evaluate microplastic pollution in terms of both environmental and human health.

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Gökkuşağı Alabalığı (*Oncorhynchus mykiss*) Mikroplastik Maruziyeti: Etkileri ve Araştırma Boşlukları

ÖZET
Dünya genelinde, bu derleme, tatlı su ekosistemlerinde önemli bir kirletici olan mikroplastiklerin, Gökkuşağı alabalığı (*Oncorhynchus mykiss*) üzerindeki etkilerini inceleyen bilimsel çalışmalar bir araya getirilmiştir. Bu çalışmalardan mikroplastik türleri, konsantrasyonları, maruziyet süreleri ve balıkların biyolojik tepkileri (dokulardaki histopatolojik değişiklikler, immün tepkiler ve biyokimyasal parametreler) gibi veriler toplanarak değerlendirilmiştir. Mikroplastikler, özellikle çiftlikte yetiştirilen alabalıkların biyolojik sistemlerinde birikmekte ve toksik etkiler yaratmaktadır. Ayrıca, mikroplastiklerin balıklarda protein, amino asit ve yağ asidi kompozisyonu üzerindeki etkileri veri setine dahil edilmiştir. Toplanan veriler SPSS programına aktarılmış ve tanımlayıcı istatistikler kullanılarak analiz edilmiştir. Mikroplastik ile bazı kirletici kombinasyonlarının ve deney sürelerinin biyolojik

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etkileri karşılaştırmalı analizlerle değerlendirilmiştir. Analizler sonucu çeşitli mikroplastik türlerinin pestisitlerle ve ağırmetallerle kombinasyonlarının balıkların dokularındaki histopatolojik ve biyokimyasal değişikliklere neden olduğu vurgulanmaktadır. Özellikle yüksek konsantrasyonlardaki mikroplastik maruziyetinin solungaç ve karaciğer gibi organların daha duyarlı olduğu belirlenmiştir. Ayrıca mikroplastik ile bazı kirletici kombinasyonların oksidatif stres, amino asit ve yağ asidi bileşimlerinde olumsuz etkiler oluşturduğu belirlenmiştir. Mikroplastiklerin balıkların biyolojik sistemleri üzerindeki etkilerine dair mevcut araştırmalardaki eksiklikleri ve laboratuvar deneylerinin, doğal ortamlarda gözlenen mikroplastiklerin çevresel dağılımını ve biyolojik etkilerini açıklayamama durumu tartışılmıştır. Özellikle laboratuvar ortamın da yapılan deneylerin doğal çevre koşullarını yeterince yansıtmadığı ve çevresel risk değerlendirmeler gibi daha gerçekçi değerlendirmelere ihtiyaç olduğu sonucuna varılmıştır. Bu sonuçlar, hem çevresel hem de insan sağlığı açısından mikroplastik kirliliğinin kapsamlı çalışmalarla değerlendirmeler yapılmasını gerektirmektedir.

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INTRODUCTION

Plastics are synthetic materials composed of long chains of molecules called polymers. Plastics are widely used in many industrial and consumer applications because they are durable, lightweight, flexible, and generally inexpensive (Andrady, 2003). However, global concerns about the widespread distribution of plastic waste and its environmental consequences are increasing. In 2017 alone, global plastic product production reached approximately 348 million tons. This is due to the widespread use of plastic products, increased production, and poor management (Wu et al., 2023). Traditionally, large pieces of plastics have been used in various industries such as product packaging, textiles, building materials, and electronic equipment, while microplastics are smaller pieces. Microplastics can be formed as a result of the breakdown of initially large pieces of plastic by environmental factors or as a byproduct of industrial production processes. Thompson et al. (2004), used the term "microplastic" to describe plastic pieces with an effective size of less than 5 mm. Also in the 1972s, researchers first identified the presence of plastic debris in coastal waters (Carpenter & Smith, 1972). Their small size allows them to be more widely distributed in the environment and penetrate various ecosystems (Barnes et al., 2009). Microplastics can have significant impacts on environmental and ecological systems and are therefore a concern to be considered.

Freshwater ecosystems contain natural resources such as lakes rivers and streams and these resources are vital to both humans and other living things. However, microplastic pollution threatens these water resources (Wright et al., 2013). Agricultural activities, wastewater treatment plants, urban runoff, and even air transport are ways for microplastics to enter these water sources (Acquah et al., 2021). This can lead to bioaccumulation in aquatic ecosystems and have detrimental effects on the health of aquatic organisms such as fish. For example, biodegradation-resistant polystyrene (PS) can accumulate in the stomach of fish (Carpenter & Smith, 1972) and be translocated through the bloodstream (Chen et al., 2006). When a living organism ingests microplastics, these particles can enter the bloodstream and reach various organs. While some of the microplastics pass through the digestive system and are eliminated from the body, some may remain in the digestive tract. Microplastic particles that accumulate in the digestive system can cause physical damage and toxic effects in this area (Teuten et al., 2009; Çağlayan & Aytan, 2020). These damages usually take the form of punctures, lesions, abrasions, blockages in the digestive system, etc.; while at the same time, they cause a decrease in the animal's hunting and a need for less food. Tissue death, slow growth rate, decrease in swimming speed and changes in genes can also be given as examples (Khalid, 2021; Ulvi, 2023). Research on microplastics in freshwater has also become increasingly important in recent years. These studies have shown the presence of microplastic pollution in various freshwater ecosystems, from rivers to lakes and from streams to dams (Lechner et al., 2014; Terzi, 2023). The majority of studies on the presence of microplastics in fish species in Türkiye have been conducted in the sea. For example; Gündoğdu et al. (2019) examined the abundance of microplastics in the stomach and digestive tracts of red mullet, grey mullet, red mullet, red mullet, horse mackerel and red shrimp species from the Marmara, Aegean and Mediterranean seas. Güven et al. (2017) reported that 34% of 1536 fish samples collected from different points along the Mediterranean coast of Türkiye contained microplastics in their stomachs and intestines.

Oncorhynchus mykiss is considered an important commercial fish species worldwide. According to the latest data, it was reported that approximately 953,000 tons of *Oncorhynchus mykiss* were produced worldwide in 2022 (FAO, 2024). Most of this production was carried out by countries such as Iran, Chile and Türkiye. In Turkey, it is grown in many inland water sources, especially in Tokat (Almus Dam), Elazığ (Keban Dam) and the Black Sea Region. According to the data of the Turkish Statistical Institute (TÜİK), rainbow trout constitutes a large part of our country's freshwater fish production. Farmed trout, which has an important place for human consumption, plays a critical role in investigating the ingestion of microplastics both to ensure food safety and to better understand the pathways of human exposure to these contaminants.

Studies on *Oncorhynchus mykiss* have investigated the negative effects of microplastics and chemical pollutants on biological systems. The researchers wanted to find out how different types and concentrations of microplastics, alone or in combination with chemicals, cause changes in tissue, immune system, biochemical parameters and oxidative stress biomarkers in fish. All of these studies were conducted using controlled laboratory experiments. However, laboratory experiments often use levels higher than the average microplastic concentrations found in the environment (Paul-Pont et al., 2018). Therefore, it is important to evaluate how accurately the results obtained in experimental environments reflect the levels of microplastic pollution in natural environments. It is also noteworthy that there are no studies on microplastic exposure in the natural habitat of *Oncorhynchus mykiss*.

In this context, we aim to identify critical knowledge gaps regarding microplastic pollution in both *Oncorhynchus mykiss* cultivation and natural populations. These studies will help to better understand microplastic flow and may also contribute to the development of strategies to reduce the impact of this global threat. For this purpose, data from existing studies on microplastic ingestion by *Oncorhynchus mykiss* were evaluated to investigate whether the amount of plastic ingestion varies according to the type of plastic, exposure time or particle size. In addition, current deficiencies in studies examining the effects of microplastics on the biological systems of fish will be determined and guiding information for future research will be provided.

MATERIAL and METHOD

The literature review was conducted in comprehensive academic databases such as Web of Science Scopus and Google Scholar. The search was conducted using keywords such as "*O. mykiss*" "microplastics" "plastic" "fish health" "aquaculture" "toxicity" and "chemical pollutants". In this study, 17 article were utilized that given an in-depth description of the microplastics used, concentration levels, experimental durations, and biological systems. Experimental studies conducted in a laboratory environment have been obtained but no studies have been found on microplastic pollution on *O. mykiss* in the natural environment.

The types of microplastics, concentrations, exposure times and responses in biological systems (e.g. histopathological changes, enzyme activities, oxidative stress indicators) reported in the selected articles are tabulated. In addition, the effects of microplastics on protein, amino acid and fatty acid composition in fish given in some studies are also added to the table.

Due to the high methodological variability across studies (e.g., testing times, tissues examined, analytical techniques), no statistically comparable meta-analysis was performed. Instead, descriptive statistical summaries such as mean values, ranges, or frequencies reported across studies were used. Graphs are presented for visual comparison of data from the literature; they do not represent original experimental data.

RESULTS

In the microplastic studies on *O. mykiss* in the literature related to different microplastic types their concentrations and the evaluation of these substances together with chemical compounds were discussed. In these studies, microplastic bioaccumulation in various tissues of fish physiological effects and changes in biomarkers have been examined in detail.

PS microplastics showed accumulation in the gill and gastrointestinal tissues of fish. Histopathological lesions and inflammation were detected in tissues especially at high concentrations. PS mikroplastics caused damage such as villus shedding and inflammatory cell infiltration in the intestine (Karbalaei et al., 2019, 2021) (Table 1).

When polystyrene microplastics were combined with chlorpyrifos (CPF) pesticide an increase in enzymatic activities was observed especially in liver kidney and muscle tissues. The combination potentiated the toxicity by increasing the effect of microplastics. Significant changes in amino acid and fatty acid compositions were detected in muscle tissue. CPF has been found to have a synergistic effect by increasing the bioavailability of microplastics (Hanachi et al., 2021, 2022). Polystyrene and polyethylene microplastics caused changes in antioxidant enzyme balance and caused histopathological damage in the liver and gills. PS-MPs together with the pesticide methiocarb were found to produce signs of neurotoxicity and oxidative stress. Long-term exposure to PE microplastics has been found to cause increases in corticosterone levels and changes in larval development (Jakubowska, 2022; Bohačková, 2023). Microplastic transfer from the packaging material to the trout fillet was observed and it was determined that it increased polymer degradation especially during high temperature applications. The presence of microplastic in fish tissue for human consumption has been calculated (6140 MP units/year) (Alak et al., 2021, 2022) (Table 1).

Table 1. Effects of microplastic and pesticide exposure on Oncorhynchus mykiss on experimental conditions, concentrations, duration, biomarkers and results of different studies

Çizelge 1. Farklı çalışmaların mikroplastik ve pestisit maruziyetinin Oncorhynchus mykiss üzerindeki; deney koşulları, konsantrasyonlar, süre, biyobelirteçlere etkileri ve sonuçları

	Experimental Conditions	Concentrations	Duration	Biomarkers and Effects	Results
Karbalaei et al. 2019	Polystyrene (PS) microplastic bioaccumulation	**PS Microplastic: 30 g/L and 300 g/L**	14 days	Gill and Gastrointestinal System: Microplastic accumulation is higher in the gill than in the gastrointestinal tract.	Microplastic accumulation in tissues increased with increasing MP concentrations. - Bioaccumulation of microplastics should be considered in environmental risk assessment. - *O. Mykiss* can be considered as a promising indicator species in monitoring environmental pollutants.
Maleki et al. 2020	PS microplastic and Chlorpyrifos (CPF)	**CPF: 2 µg/L 6 µg/L PS Microplastic: 30 µg/L 300 µg/L** **Combination: 30 µg/L MP + 2 µg/L CPF**		SOD Activity in Liver Kidney and Muscle Tissue: The combination of 30 µg/L microplastic and 2 µg/L chlorpyrifos decreased enzyme activity.	The presence of microplastics decreased the bioavailability of chlorpyrifos in the liver and kidney while increasing the toxicity and bioavailability of chlorpyrifos in muscle tissue.
Alak et al. 2022	Trout fillet with sous-vide technique	**7 different temperatures** **> 65°C 67% <50 µm 8% 500–1000 µm**	3 different durations	Presence size shape polymer types and human intake level of microplastics (MP)	1.27 ± 0.54 MP/g was found in 1 g of fish tissue. Human intake level was estimated to be at least 6140 MP units/year. High temperature applications increased polymer degradation.
Hanachi et al. 2021	PS Microplastics CPF PS Microplastics +CPF Combination	**30 µg/L 300 µg/L** **2 µg/L 6 µg/L** **30 µg/L PS Mikroplastic + 2 µg/L CPF 300 µg/L PS-MP + 6 µg/L CPF** **Combination: 300 µg/L mikroplastic + 30 µg/L klorpirifos**	96 hours	Minimal amino acid and fatty acid changes in muscle tissue no effect on protein content Minimal changes in amino acid and fatty acid composition no effect on protein content Significant changes in amino acid and fatty acid composition significant decrease in protein content	PS-MPs showed low effect on nutritional parameters in *O. mykiss* muscle tissue. No significant effect was observed on protein content. CPF alone did not cause any significant effect on nutritional parameters in muscle tissue. Combined PS-MP and CPF caused significant decreases in amino acid fatty acid and protein contents in muscle tissue. CPF significantly enhanced the effects of PS-MPs. In muscle tissue the combination of 300 µg/L microplastic and 30 µg/L chlorpyrifos increased SOD activity.
Karbalaei et al. 2021	PS-Microplastic CPF PS-Microplastic + CPF Combination	**30 µg/L 300 µg/L** **2 µg/L 6 µg/L** **30 µg/L PS-MP + 2 µg/L CPF 300 µg/L PS-MP + 6 µg/L CPF**	96 hours	Histopathological changes in fish gills intestinal villus shedding inflammatory cell infiltration Histopathological lesions in muscle tissue inflammatory cell infiltration in the intestine villus shedding. Severe histopathological changes in the gills and intestine; massive necrosis in the intestine	PS-MPs caused toxicity in *O. mykiss.* Histopathological lesions were significantly increased at high PS-MP concentrations. It has been determined that CPF alone causes histopathological lesions in fish tissues. The combined effects of PS-MPs and CPF produced severe histopathological lesions in the intestinal and gill tissue. CPF enhanced the effect of PS-MPs.
Hodkovicova et al. 2021	Pure polyethylene (PE) microparticles	**%05 %2 %5**	6 weeks exposure	Hematological profile lipid peroxidation in gills histopathological examination biochemical profile	PE microparticles may affect the health indexes of *O. mykiss*; potential issues for human consumption.

Alak et al. 2021	Different packaging methods (groups S and C) and storage temperatures (+4 °C and -20 °C)	Polystyrene plate + wrapped film (S) Chitosan film + Polystyrene plate + wrapped film (C)	21 days	pH TBARS TVB-N MP presence (shape size polymer type) ATR-FTIR analysis	The presence of MP was detected most in group S and least in group C. The chemical quality was better at -20 °C but the presence of MPs was found to be higher at this temperature.
Zwollo et al. 2021	PS microplastic exposure in primary cultures obtained from the anterior kidney of *Oncorhynchus mykiss*	**0.8–20 µm PS microbeads**	-	Flow cytometry RT-qPCR RAG1 gene expression immunoglobulin heavy chains mi and tau	PS microplastics reduced the abundance of non-phagocytic developing B cells. It was associated with decreased RAG1 gene expression and immunoglobulin heavy chains. Microplastics can negatively impact antibody responses and B cell development.
Banihashemi et al. 2021	Exposed to *Yersinia ruckeri* (5% and 10% LD50) and microplastics (500 and 1000 mg/kg) for 30 days	**5% 10% LD50 *Y. ruckeri* 500 and 1000 mg/Kg MPs**	30 days	Blood biochemical parameters hepatic oxidative biomarkers (catalase superoxide dismutase malondialdehyde glutathione peroxidase)	Co-exposure to *Y. ruckeri* and MPs caused significant changes in blood biochemical parameters and hepatic oxidative biomarkers. Combination exposure showed synergistic effects on clinical parameters in fish.
Jakubowska et al. 2022	From eye stage embryos to motile larvae and pre-production pellets (**3000 µm**; PS and PE)	PS, PE	69 days	Corticosterone concentration genotoxicity cytotoxicity	Higher corticosterone in larvae in PE treatment increased genotoxicity with exposure to PS pellets; no change in cytotoxicity was noted.
	PE Microspheres (**150–180 µm**; PE)	PE	29 days (short time)	Microparticle ingestion yolk depletion	Microparticle ingestion was observed in up to 24% of larvae; No change in height gain was observed Rapid depletion of yolk sac after PE treatment.
	PE treatment of salmonid larvae	PE	69 days (long time)	Development and growth parameters	After long-term exposure after PE treatment the size of larvae did not differ from control but the rate of yolk depletion varied.
Hanachi et al. 2022	Exposed to CPF (2 and 6 µg/L) and PS microplastic (30 and 300 µg/L)	**2 and 6 µg/L CPF 30 and 300 µg/L PS-MPs**	-	Catalase (CAT) glutathione peroxidase (GPx) glutathione S-transferase (GST) activities in liver and kidney tissues	The combination of MPs and CPF resulted in significant changes in enzymatic activities in different tissue samples. While MPs increased GPx and CAT activity in the kidney GST activity in the liver also showed a significant increase. These effects increased the bioavailability of the pollutants.
Banihashemi et al. 2022	*Yersinia ruckeri* and microplastics Effect on *Oncorhynchus mykiss*	***Y. ruckeri*: 5% and 10% of LD50 - MP: 500 and 1000 mg/kg**	30 days	Blood Biochemistry: MPs caused increases in creatinine glucose triglyceride levels. - Liver Oxidative Stress: SOD activity and MDA content increased. - Clinical Parameters: The combination of *Y. ruckeri* and MPs decreased total protein albumin globulin levels.	When MPs and *Y. ruckeri* were exposed together a synergistic effect on clinical parameters was observed. - The presence of MPs worsened *Y. ruckeri* infection and increased the oxidative stress level of fish.
Bohačková et al. 2023	Food-sourced PE	**%0,5, %2, %5**	6 weeks	Hematological profile liver and gill histopathology ROS formation	Decrease in hematocrit hepatodystrophies in the liver signs of inflammation and damage in the gills
	Microparticle size	**<5 µm**		The intestine was examined with an electron microscope	PE particles became smaller than 5 µm in the intestine and were able to reach the tissues.
	Antioxidant Enzyme Capacity	-		Lipid peroxidation imbalance in antioxidant capacity	Inflammation and gill damage at high concentrations of PE; increased antioxidant capacity
	Immunity and Cytokines	-		Proinflammatory genes cytokine expression	Increase in proinflammatory genes in the liver in 2% PE Proinflammatory cytokine

					expression in kidneys
Hollerova et al. 2023	*O. mykiss* fry 0.5% 2% 5% PS microplastic diet concentrations	**%0,5, %2, %5**	6 weeks	Neutrophil segment count lymphocyte count plasma ammonia ceruloplasmin	Immune response at 5% PS-MP concentration decrease in plasma ammonia in all groups liver and gill damage Disruption of antioxidant balance at 5% concentration.
Alak et al. 2023	*O. mykiss* microplastic load	**300–500 µm, 500–800 µm,800–1200 µm,1 mm, 2mm, 3mm, 4mm, 5mm**	Egg Fertilized egg eyed egg of the flame floating fry	MP amounts microplastic colors and polymer types in feed groups	14 MPs were detected in the stages where no bait was thrown; 557% increase in fish that started external feeding (92 MP). In black and blue Dominant microplastics of polyethylene type. External source inputs It may cause MP contamination during fish production and processing.
Baker, 2023	Effects of PS microplastics on juvenile trout.	**Low PS microplastic: 10 µg/L**	acute exposure period	Behavioral Changes: Behavioral abnormalities after microplastic exposure. - Intestinal Tissue Damage. Oxidative Stress: Increased levels of oxidative stress	High microplastic exposure caused rapid deaths. Even at low levels polystyrene causes behavioral disorders and damage to intestinal tissue.
Banaee et al. 2023	Enrofloxacin (ENR)	**0 1.35 ml/kg 2.7 ml/kg (with diet)**	21 days	Increased enzymatic activity in all biomarkers except GGT Changes in triglyceride cholesterol glucose urea creatinine total protein and albumin levels.	ENR caused significant changes in blood biochemical values. An increase in enzymatic activities was observed especially at high concentrations.
	Microplastics	**0, 1000 mg/kg 2000 mg/kg (with diet)**		Liver enzymes: increased levels of SOD MDA G6PDH; Decrease in CAT and GPx levels; decrease in total antioxidant (ANT) levels.	MPs induced significant changes in liver enzyme levelsIt was observed that oxidative stress increased while cellular antioxidant levels decreased.
	ENR + MP Combination	**1.35 ml/kg ENR + 1000 mg/kg MP 2.7 ml/kg ENR + 2000 mg/kg MP**		Synergistic effects on all biomarkers in the combination of ENR and MP; significant changes in blood biochemistry and liver enzymes.	It increased the toxicity of ENR causing more pronounced physiological and biochemical changes. Significant changes were observed especially in enzymes affecting liver functions. MPs synergistically increased the bioavailability and toxicity of ENR.

"Data compiled from 17 peer-reviewed articles. All values are literature-derived."

Co-exposure to microplastics and Yersinia ruckeri, a bacterial species that causes infections in fish, caused significant changes in blood biochemistry and oxidative stress biomarkers. The presence of microplastics showed a synergistic effect on clinical parameters in fish by worsening *Y. ruckeri* infection (Banihashemi et al., 2021, 2022). The effects of microplastics on the immune system are also noteworthy. In particular it negatively affected B cell development reduced antibody responses and increased proinflammatory gene expression. They found that high concentrations of PS microplastics had negative effects on antioxidant balance and caused damage to liver tissue (Zwollo, 2021; Hollerova, 2023) (Table 1).

According to the reviewed literature, tissue-specific changes in the duration of microplastic exposure in *Oncorhynchus mykiss* are evident. In particular, short-term exposure appears to have only limited effects on the gills, while long-term exposure appears to cause more pronounced physiological effects on the liver and muscle tissue (Karbalaei et al., 2021; Banihashemi et al., 2022).

1.Bioaccumulation of Polystyrene Microplastics in Gills and Gastrointestinal System

When polystyrene microplastic accumulation in the gill tissue of *Oncorhynchus mykiss* was compared with the accumulation in the gastrointestinal tract, higher accumulation was detected in the gill tissue (Karbalaei et al., 2019), suggesting that gill tissue is more vulnerable to environmental microplastic accumulation, probably due to direct contact with water.

2.Effect of Chlorpyrifos and Polystyrene Microplastic Combination on the Liver

Findings in the literature (Maleki et al., 2020; Hanachi et al., 2022) show that co-exposure of chlorpyrifos and PS microplastics leads to an increase in oxidative stress biomarkers such as superoxide dismutase in liver tissue (Figure 1). This can be said to mutually increase the bioavailability of the combination and more significantly affect oxidative stress mechanisms in liver tissue.

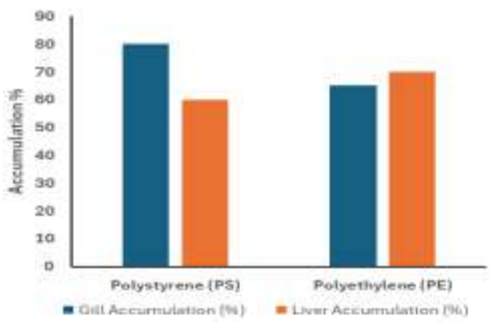

Figure 1. Accumulation rates of different microplastic types in O. mykiss (All data shown are compiled from previously published studies. These figures are based on literature-derived values and used for illustrative purposes only. No original experimental data were generated in this study.)

Şekil 1. Farklı mikroplastik türlerinin *O. mykiss*'deki birikim oranları (Tüm veriler daha önce yayımlanmış literatür çalışmalarından derlenmiştir. Şekil, yalnızca görsel karşılaştırma amacıyla hazırlanmıştır; orijinal deneysel veri içermemektedir.)

3.Effect of Polystyrene Microplastics on Muscle Tissue

At low concentrations, PS microplastics did not cause significant changes in the amino acid and fatty acid composition of muscle tissue (Hanachi et al., 2021). However, at high concentrations, significant changes in these components were observed and a decrease in tissue nutritional quality was reported, suggesting a dose-dependent effect on muscle physiology (Figure 2).

4.Neurotoxic Effects of Methiocarb and Polystyrene Microplastics

Although studies on the effects of the combination of PS microplastics with methiocarb pesticides are limited, some results suggest that this combination may affect neurotoxic biomarkers such as acetylcholinesterase (AChE) activity. However, it is still unclear whether this effect is additive or synergistic, and the effects vary depending on the duration of exposure and dose (Hanachi et al., 2022).

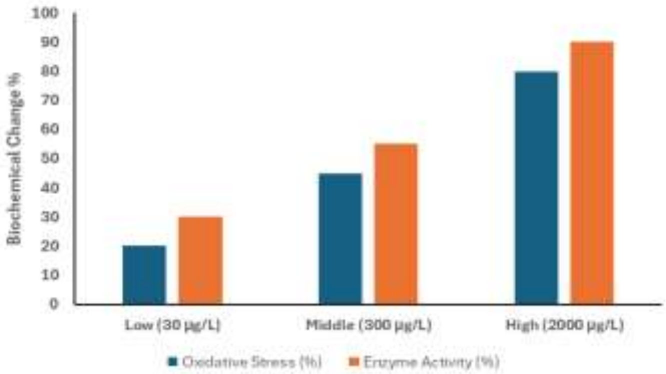

Figure 2. Effects of different microplastic concentrations on oxidative stress and enzyme activity (Data represent a synthesis of findings from multiple published studies. These visualizations are descriptive and not based on original measurements. Statistical comparisons are illustrative and based on literature-derived values.)

Şekil 2. Farklı mikroplastik konsantrasyonlarının oksidatif stres ve enzim aktivitesi üzerine etkileri (Veriler literatürden derlenmiş çalışmalara dayanmaktadır. Şekil yalnızca betimleyici amaçla sunulmuş olup, özgün deneysel veri içermemektedir. İstatistiksel karşılaştırmalar yalnızca temsili amaçlıdır.)

DISCUSSION

No difference was determined in the types of microplastics used in the studies. It was determined that mostly polystyrene and polyethylene microplastics were preferred, and microplastic types such as polyvinyl chloride (PVC) or polylactic acid (PLA) were not examined sufficiently. As a result of the analysis of the collected data, it was determined that PS microplastics accumulated more in the gills and gastrointestinal system and caused oxidative stress. PE was observed to affect the liver and hematological profile in particular. Other studies have shown that PS microplastics accumulate in the gills and digestive systems of organisms such as blue mussels, zebrafish and nematodes (Magara, 2018; Qioa, 2019; Yu, 2020; Kaliyoanni, 2021). Gündoğdu et al. (2019) detected microplastics (polyethylene, polypropylene, polyethylene terephthalate) in the stomach and digestive tracts of 44.3% of fish collected from the Marmara, Aegean and Mediterranean regions and reported that an average of 1.08 microplastics were found per fish. Gündoğdu et al. (2020) detected 283 microplastic particles in the gastrointestinal tract of 243 fish belonging to five species, and an average of 1.1 microplastics were found per fish. The frequency of microplastic occurrence was determined as 61.6% in the Aegean Sea, 40.5% in the Marmara Sea, and 39.2% in the Mediterranean. Similarly, Terzi (2023) reported the individual mean microplastic abundance as 2.87 ± 0.27 MP/individual and 0.014 ± 0.001 MP/g in grey mullet obtained from Lake Cernek in the Kızılırmak Delta. It has been determined that PVC and PLA affect the growth of aquatic organisms (fish, phytoplankton, invertebrates, zooplankton) and cause oxidative stress (Zhao, 2019; Xia, 2020; Chagans, 2021). In *O. mykiss* and other species, the routes by which microplastics enter biological tissues (ingestion, absorption through the gills), their residence time in the digestive system and their metabolic effects also vary (Yuan, 2019; Li, 2020). Although microplastics are usually present in the gills for a short time, they can remain in the gastrointestinal tract for a long time (EFSA, 2016).

Studies show that the effects of microplastic types on toxicity need to be investigated further. In general, the effects of the morphology of microplastics, i.e. their shape, color and size, on toxicity have been investigated. (Qiao et al., 2019) In a study examining the effects of three different forms of microplastics (fiber, piece, bead) on intestinal toxicity in zebrafish, they stated that fiber pieces were more effective. Another study examined the effects of fiber and granule-shaped microplastics on copepods and determined that exposure to fibers reduced the rate of algae ingestion (Cole et al., 2019). It was observed that studies examining microplastic effects on *O. mykiss* did not focus on the types of microplastics and the effects of morphological changes.

Microplastic pollution has been widely detected in commercial fish species. Studies have shown that the presence of microplastics in commercial fish species such as European anchovy (*Engraulis encrasicolus*), *Sardina pilchardus*, and European whiting (*Merluccius merluccius*) is mostly fiber particles compared to other types of microplastics (Avio, 2015; Compa, 2018; Bakır, 2020). Microfibers have been found in high density in the digestive systems of farmed fish such as European sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) (Sánchez-Almeida et al., 2022). Differences between *O. mykiss* and commercial fish can be explained by feeding strategies, habitat differences, and the distribution of microplastics in the environment. Filter-feeding species (anchovies, sardines, etc.) accumulate more microplastics, while bottom-feeding fish (merschela, mullet, etc.) are exposed to higher levels of microplastics from sediment (Blettler et al., 2019). Predatory fish such as *O. mykiss* may be exposed to microplastics through their prey (Azizi et al., 2021). The amount of microplastics was found to be higher in aquaculture fish compared to wild-caught fish and fish purchased from the market. The main reasons for this are thought to be the use of plastic materials such as tanks, nets, cages, buckets and scoops used in aquaculture (Zhou et al., 2021). In addition, feeds and fish meal used as fish food in aquaculture have been cited as sources of microplastic pollution (Wright et al., 2013), and it has been reported that fish meal contains 124 microplastics per kg, including a large number of microfibers (Thiele et al., 2021). It has been mentioned that comparisons of microplastic amounts in fish species cultivated in different habitats in microplastic studies may distort the results because these studies do not distinguish whether the amounts of plastic come from the natural environment or from aquaculture, and therefore it is not considered healthy to compare high amounts of microplastics in fish species. It is not fully known whether microplastics are directly transmitted from pollution in the aquatic environment or during the fishing process (Wu et al., 2023). *O. mykiss* is an important freshwater aquaculture species worldwide, and the impact of microplastics should be assessed in this context. In aquaculture, microplastic accumulation is influenced not only by feed, but also by factors such as equipment used and water quality. The contribution of feeds and systems used in commercial *O. mykiss* farming to microplastic contamination is not yet well understood. Future studies should evaluate the impact of microplastics on *O. mykiss* in different culture systems compared to natural populations.

The differences between the microplastic concentrations used in the studies were found to be significant. Experiments were generally conducted between low (30 μg/L - 1000 mg/kg) and high (300 μg/L - 2000 mg/kg) concentrations. Karbalaei et al. (2021) showed that high concentrations of plastics had a significant effect on tissues such as muscle and liver, while low concentrations indicated slight accumulation in gill tissues. In a meta-analysis study that compiled studies on microplastics in fish species distributed in freshwater, it was stated that there was variability in the amount of microplastics among the species examined, with the lowest amount of microplastics being 0.153 per individual in the *Macrodon ancylodon* species, and the highest amount of microplastics being 40,880 per individual in the *Prochilodus lineatus* species. This difference is associated with the pollution of water resources and the feeding strategies of the species (Azizi et al., 2021). It is also known that fish with a detrivorous feeding style, such as *P. lineatus*, consume more microplastics in their environment (Blettler et al., 2019). Differences in microplastic accumulation among fish generally depend on factors such as their feeding habits such as swallowing, filtering, suction feeding, habitat, and intestinal structures (Yuan, 2019; Li, 2020).

Microplastics are mostly taken into the body of fish through swallowing and gills. Microplastics usually remain in the gills for a short time, and those that are too large to enter the spaces between the gill filaments may remain on the gill surface and can then be easily cleaned. Microplastics can remain in the gastrointestinal tract of fish for a long time. It is known that the tortuous structures in the fish's intestines affect the amount of accumulation and the duration of microplastics in the body. In light of this information, it is seen that it is important to understand the ways in which microplastics enter the fish's body and their detectability. These findings show that these variables are not taken into account much in microplastic studies on trout.

The average plastic load in the Danube River was determined as 4664.6 pieces per 1000 m^3 (Lechner et al., 2014). In a study conducted in the Rhine River in Europe, the average microplastic density was determined to be 892,777 particles/km^2 in samples taken from 11 different points in an area of 820 km (Mani et al., 2015). Another study in China examined the formation and distribution of microplastics in surface waters at the Three Gorges Dam. Samples from the dam detected between 3,407.7 x 10^3 and 13,617.5 x 10^3 microplastic particles per kilometer in the mainland Yangtze River. Among the microplastic types, polyethylene, polypropylene and polystyrene were found (Zhang et al., 2015). In another study conducted in the Three Gorges Reservoir, microplastic pollution in surface waters and sediments was examined. The average microplastic density in surface waters was found to be 4703 ± 2816 n/m3 and it was determined that the highest pollution was in urban centers (Di & Wang 2017). As seen in the studies, the pollution levels of water basins are increasing due to reasons such as urbanization, high population, and industrialization in the environment. These findings show that the pollution status of the habitats where microplastics are detected should be evaluated together, taking into account the biological characteristics of fish species and the pollution levels in water resources. All of the research in this study was conducted in a laboratory environment and was carried out without considering these outcomes in the natural environment. It is seen that more studies are needed in aquatic ecosystems for microplastic uptake by trout and their evaluation.

Karbalaei et al. (2019) found that plastics alone did not cause significant changes in these biomarkers. The combination of plastics with other toxic substances, especially pesticides, produced significant synergistic effects. In particular, as seen in the studies of Hanachi et al. (2021) and Banihashemi et al. (2022), plastics together with pesticides showed both oxidative stress and tissue damage-enhancing effects. In most studies, serious histopathological changes were observed in the gills and intestines of fish exposed to plastics. For example, significant damage was observed in the intestines of fish (*Dicentrarchus labrax*) exposed to PVC plastics (Peda et al., 2016), and in another study, the gills of European sea bass were determined to be the organs most affected histologically by mycoplastics (hyperemia, hypertrophy, hyperplasia and necrosis) (Saraiva et al., 2015). This highlights that plastics are an important factor to consider in environmental risk assessment, particularly in the studies by Karbalaei et al. (2021) and Baker (2023), where intestinal tissue damage is evident. Plastics and their combination with pesticides caused significant changes in oxidative stress and enzyme activities in liver and muscle tissue. Changes in enzymes such as SOD, CAT and GPx indicate that fish are under metabolic stress. Similar results have been reported in carp, tilipya and zebrafish species that microplastics cause oxidative stress (Subaramaniyam et al., 2023). In addition, it has been determined that the number of studies conducted on commercially important and threatened species is limited (Hollerova et al., 2023). The small number of studies conducted on commercially important and threatened species is an important deficiency in terms of developing strategies for the conservation of these species (Hodkovicova et al., 2021). These deficiencies identified in this study provide important clues to guide future studies. In this context, trout species (such as *Oncorhynchus mykiss*) have not been studied sufficiently in the field in terms of mycoplastic exposure despite their commercial importance. The limited number of microplastic-based field studies on trout is thought to be due to the fact that these species are often grown in controlled aquaculture systems and the difficulties in monitoring their natural populations. In addition, trout may have less research interest due to the fact that they mostly live in inland water systems and are found in systems that are less monitored and reported compared to marine environments. Therefore, interdisciplinary field studies on both cultured and natural trout populations are a critical need to fill this important gap in the literature.

Alak et al. (2021) also determined that different food processing methods affected the presence of microplastics in fish tissues. However, the potential effects of microplastics and nanoplastics on human health and how and in what quantities plastics are ingested are not yet fully known. Current information suggests that microplastics can be carried through the food chain and reach humans. Özsoy et al. (2024) reported the presence of microplastics in human stomach contents. However, most of the studies are on marine organisms and it has been determined that microplastics accumulate in large quantities in the gastrointestinal systems of marine organisms. The digestive systems of marine organisms are discarded before consumption but some shelled aquatic animals such as mussels are consumed with their digestive systems. It is known that microplastics smaller than 150 µm pass through the intestinal epithelium and accumulate in other organs (EFSA, 2016). Therefore, data on the degradation and excretion of microplastics in the digestive system and the formation of microplastics and nanoplastics in food processing are a major gap in the literature.

This article identifies some important gaps in the analysis of studies on the effects of microplastics on the biological systems of trout. One of these gaps is that many studies have been conducted only in laboratory settings and do not adequately reflect the levels of microplastic pollution observed in natural environments (Lenz, 2016; Paul-Pont, 2018). The data obtained showed that the microplastic concentrations used in experimental conditions were generally higher than those in environmental conditions. The types and concentrations of microplastics used in laboratory settings do not match the levels to which fish are exposed in the natural environment (Khalid et al., 2021). This creates limitations in fully understanding the effects of microplastics on fish health. The differences between the effects of environmental microplastic pollution and microplastics used in laboratory environments reveal the need for more realistic environmental risk assessments (Acquah et al., 2021). Additionally, it was found that the number of studies on commercially important and threatened species was limited (Hollerova et al., 2023). The low number of studies on commercially important and threatened species is an important shortcoming in developing strategies for the protection of these species (Hodkovicova et al., 2021). These deficiencies identified in this study provide important clues to guide future studies.

Gaps in studies on the effects of microplastics on the biological systems of fish draw attention in some important areas:

1. Microplastic Types and Concentrations: Most existing research focuses on specific types of microplastics and does not adequately examine the effects of different concentrations of these types. While some studies have only observed common types of microplastics, such as polystyrene and polyethylene, other potentially harmful species have been overlooked (Khalid et al., 2021).
2. Diversity of Biological Responses: The effects on the biological systems of fish are limited to histological changes and other important biological responses such as the immune system biochemical parameters and behavioral responses are not sufficiently investigated. This creates an important knowledge gap for a comprehensive assessment of microplastic exposure (Hollerova et al., 2023).
3. Impact of Ecological and Physical Conditions: Experimental studies are often conducted in controlled laboratory environments ignoring how ecological and physical conditions (such as temperature pH water quality) alter the effects of microplastics on fish. Failure to take these variables into account in natural environments reduces the applicability of the results obtained (Lenz et al., 2016).

CONCLUSION

In this study gaps in the existing literature on the effects of microplastics on fish biological systems were identified and recommendations were presented to guide future research. It has been observed that most experimental studies have been conducted using concentrations and types of microplastics that differ from actual environmental conditions. This creates significant limitations in generalizing laboratory findings to natural settings. The differences between environmental microplastic pollution and the effects of microplastics used in laboratory environments reveal that more realistic environmental risk assessments need to be made. In addition the small number of studies on commercially important and threatened species is an important deficiency in developing strategies for the protection of these species.

In summary the synergistic effects of microplastics and pesticides on fish cause serious problems in the ecosystem and it is of great importance to evaluate the potential effects of these pollutants on human consumption. As the last link in the food chain humans' potential exposure to these pollutants may lead to increased health risks in the future. Therefore further investigation of the environmental and physiological effects of microplastics and understanding the long-term effects of their combined exposure to pesticides on the ecosystem is a critical issue for both environmental and human health.

These deficiencies identified in this study provide important clues to guide future studies. In particular larger-scale and diverse-species studies are needed to better understand the effects of microplastics on fish. In conclusion in this study the effects of microplastics on *O. mykiss* were examined and it was evaluated how accurately the experimental studies reflected natural conditions. The findings reveal that more realistic and comprehensive studies on both environmental and commercial fish species need to be conducted in the future.

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Contribution Rate Statement Summary of Researchers

Concept: Ölmez, A., Demir, H.; Design: Ölmez, A.; Data Collection or Processing: Ölmez, A. Demir, H.; Statistical

Analyses: Ölmez, A. Demir, H.; Literature Search: Ölmez, A, Demir, H.; Writing, Review and Editing: Ölmez, A., Demir, H.

Conflict of Interest

The authors declare that there is no conflict of interest among them.

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