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Interaction of Microplastic Presence and Oxidative Stress in Freshwater Fish: A Regional Scale Research, East Anatolia of Türkiye (Erzurum & Erzincan & Bingöl)

Muhammed Atamanalp [1], Mine Kokturk [2], Mahinur Kırıcı [3], Arzu Ucar [1,*], Muammer Kırıcı [4], Veysel Parlak [5], Ahmet Aydın [6] and Gonca Alak [7]

1 Department of Aquaculture, Faculty of Fisheries, Ataturk University, Erzurum 25240, Türkiye
2 Department of Organic Agriculture Management, Faculty of Applied Sciences, Igdir University, Igdir 76002, Türkiye
3 Department of Chemistry, Faculty of Arts and Science, Bingöl University, Bingöl 12000, Türkiye
4 Department of Veterinary Health, Food Agriculture and Livestock Vocational School, Bingöl University, Bingöl 12000, Türkiye
5 Department of Basic Sciences, Faculty of Fisheries, Ataturk University, Erzurum 25240, Türkiye
6 Department of Plant and Animal Production, Finike Vocational School, Akdeniz University, Antalya 07058, Türkiye
7 Department of Seafood Processing Technology, Faculty of Fisheries, Ataturk University, Erzurum 25240, Türkiye
* Correspondence: arzuucar@atauni.edu.tr

Abstract: The presence of microplastic (MP) in different fish species taken from stations in Erzurum, Erzincan and Bingöl was examined. The obtained data were classified and shared with the scientific world as the first record made in this region. In the obtained results, the most dominant color was black (39–58%) and the most prevalent forms were fragment and fiber. The sizes (0–50, 50–100 µm) of microplastics differed according to the region and species. When the number of MPs in the gastrointestinal systems of different fish species in the Bingöl, Erzurum and Erzincan provinces was evaluated, the most microplastics were found in *Squalius squalus* (20.7%) and *Blicca bjoerkna* (18.2%) in Bingöl province from among six different species. In Erzincan province, four fish species were sampled, and the rates were (29.7%) in *Capoeta umbla* and (26.6%) in *Blicca bjoerkna*. The highest abundance in Erzurum province was determined in *Cyprinus carpio* (53.0%). In the analyses performed on liver tissues, the highest ROS, which is the indicator of oxidative damage, was listed as Bingöl > Erzincan > Erzurum, while MDA levels were recorded as Bingöl > Erzurum > Erzincan, from high to low. When the differences between species were examined, the highest SOD and CAT activity was determined in the *Mugil cephalus* species. Considering the total MP numbers in fish samples, 47 MP was determined in this species. On the other hand, in the *Squalius squalus* species, where the highest total MP was determined, SOD and CAT activities were found to be low in Bingöl province. Therewithal, the high levels of ROS and MDA in this species can be said to induce oxidative stress due to the presence of microplastics on the one hand and to reduce antioxidant levels on the other hand. When the findings were evaluated, it was concluded that MPs in freshwater are a potential stressor, and freshwater environments may represent a critical target habitat for future MP removal and remediation strategies.

Keywords: microplastics; fish; oxidative stress; microplastic presence

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1. Introduction

Pollution of marine/freshwater mediums, especially with microplastics (MP), is a worldwide ecological issue that raises academic concerns. This situation has spurred a considerable number of studies that focus on the formation of MP, its interference with chemical contaminants, its uptake by fish, and the adverse effects [1,2]. While research

on MP contamination has been carried out especially on marine ecosystems, research on freshwater mediums is scarce [2,3].

Determination of MP contamination in freshwater is very important because of; (i) high biodiversity of freshwater ecosystems threatened by human-induced stressors [4] (ii) people's dependence on fresh water for a range of ecosystem services, including food and drinking water, which poses a human health risk (iii) river transport to the world's oceans [5] being recognized as the main source of plastics in the seas [6,7].

It is assumed that some (if not all) of the microplastics entering rivers will be floating and will be easily transported downstream. Because sources of MPs are anthropogenic, populated or industrial areas with high flow input are likely to contain more MPs than areas subject to little anthropogenic input [8–10]. It had been reported that MP pollution may have more negative consequences in lake and river ecosystems as closed river basins act as a source [11]. MPs found in fish guts can be considered a representation of MP contamination in rivers, as some of the MPs in the environment are likely to be contained in the biota [12,13]. There is a significant discrepancy between reports on plastics in freshwater and marine systems, with only about 4% of studies on microplastics reported for freshwater [3].

It is known that ingestion of MPs by marine organisms can cause obstruction of the gastrointestinal tract or inflammatory responses resulting in a range of adverse effects such as lower energy reserves, reduced reproduction/growth, oxidative damage, metabolism disruption, and cellular lesions. Among the limited information on freshwater fish, Rochman et al. [14] reported that exposure to polyethylene-MPs (PE-MPs) induces hepatic stress, including glycogen depletion, fatty vacuolization, and cell necrosis in Japanese medaka (*Oryzias latipes*). It had also been reported that bioaccumulation of PS-MPs can disrupt lipid and energy metabolism and can also induce oxidative stress in zebrafish (*D. rerio*) liver [15]. These studies showed that the bioaccumulation of MPs can induce toxic effects in different freshwater fish species, and this should be carefully evaluated.

Since fish are representative groups of aquatic ecosystems, they are considered to be the common biological model used to determine the toxicity of MPs [16]. As very fine-sized microplastics pass through the gastrointestinal tract, they can be absorbed from the gut, displaced, and threaten survival as they enter the bloodstream [17]. Although MPs generally accumulate in the gastrointestinal tract of fish, they can also be transported to the liver and other organs. In order to detect organs with high MP toxicity and evaluate the mechanisms involved, it is important to identify the main MPs accumulated in fish organs following MP exposure [18]. Fish MP absorption can occur orally, through the gills and skin, and can accumulate rapidly in the gills and intestines [19]. Long-term MP exposure in fish can induce an inflammatory response in the gut, which can lead to metabolic disorders and diseases due to intestinal imbalance and damage to the microbial composition [20].

Rivers are dynamic mediums and can be subject to the accumulation of external inputs such as wastewater inputs and litter, and agricultural runoff, which contributes to the MP load [10,21]. However, research so far has not fully elucidated the extent to which freshwater fish ingest MPs, the variety of complex influences that can affect ingestion, and the potential impact on ecosystems. This study was designed to elucidate the action mechanism, stress response, damage and metabolic disorders caused by MP and MP uptake in freshwater fish in Turkey (Erzurum, Erzincan, Bingöl) as preliminary data for future studies. The study will use the following method:

1. Determination of MP abundance and characteristics in dominant fish species at the determined stations;
2. Evaluation and characterization of MPs found in gastrointestinal system (GIS);
3. Determination of the effect of MP presence on oxidative damage in liver tissues by evaluation of oxidative stress marker levels.

2. Materials and Methods

2.1. Fish Sampling

Fish samplings were made with Smith Root LR-24 Electrofisher at the determined stations in 3 different cities' (Erzurum, Erzincan and Bingöl) streams (Figure 1). They were classified according to their species and weight and brought to the laboratories with laptap coolers supported by ice cassettes. Inland Fish Species Identification Key was used for the determination of the caught fish species (at the necessary count to ensure reliability in the statistical analysis).

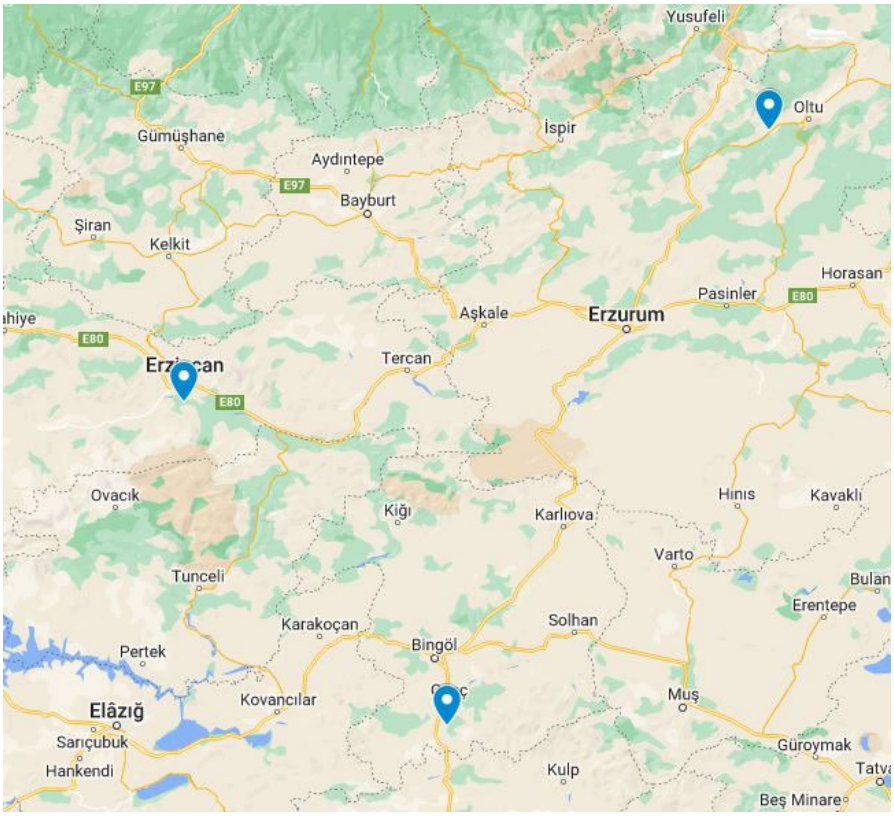


Figure 1. Research area, sampling stations in Erzurum, Erzincan and Bayburt provinces.

2.2. Laboratory Studies

Contamination control Care was taken to use non-plastic glass/aluminum/chrome and steel materials to prevent contamination of the equipment used at all stages, from hunting to sampling in field studies. Sampled fish species from the stations were brought to Atatürk University Faculty of Fisheries Laboratories in coolers and kept in a deep freezer (−20 °C) until analysis. After determining the morphological features of the fish samples (such as length, weight, species determination, and intestinal weights), samples were taken from all species for the incidence of MP intake level, the gastrointestinal tract was removed in these groups, and they were weighed and transferred to beakers [22].

In order to prevent MP contamination from a different source during the samples' preparation, the researchers used cotton lab coats and nitrile gloves at all stages of the process. All work surfaces and dissection materials were cleaned with 70% ethanol and used, and after the dissection process, the fish were washed 2 times with ultrapure water [23]. In all procedures, 6 clean glass Petri dishes were placed next to the work area and analyzed as procedure blank controls. In intestinal samples, 6 operational cavities containing ultrapure water (to evaluate possible laboratory contamination that may have occurred during the digestive procedures despite all care) were analyzed in place of the fish sample, without tissues, in parallel [24].

2.3. Fish Sampling Analyses

Preparation of gastrointestinal tissues for microplastic analysis: The gastrointestinal tissues dissected from the sampled fish were weighed and placed in 800 mL glass bottles, 30 mL of KOH (10%, V/V) was added to 1 g of tissue for each fish digestive system, and the mouths of the glass bottles were covered with aluminum foil. Digestive samples were kept in a shaking incubator at 60 °C for 48 h to digest organic material [23–25]. At the end of the incubation period, the entire homogenate was vacuum filtered through glass-microfiber filter membranes (pore size 1.2 µm). The filters were placed in glass Petri dishes and dried for 24 h (40 °C) [26].

MP characterization and identification: A visual evaluation of the items photographed in the stereomicroscope, including maximum length, color and shape, was made. At this stage, the longest or widest dimensions of each particle were also included in the closest group [22]. The MP morphotypes identified in the species as a result of the sampling were color (blue, black, gray/whitish, yellow, red/pink, green), shape (fragments-irregular pieces; lumps- spherical and oval markets; fibers- thin and long pieces) and size (<200 µm, 201–300 µm, 301–500 µm, 501–1000 µm, 1001–2000 µm, 2001–3000 µm and 3001–5000 µm) using a software program (ImageJ). Polymer identification from the gastrointestinal tract was made using the Fourier Transform Infrared Spectroscopy (Fourier-transform infrared spectroscopy, FT-IR), which is widely used due to its economy of particles per individual [24,27].

Homogenate preparation: The livers were homogenized at 3 times their weight with phosphate buffer and centrifuged at 13,000 rpm and biochemical analyses were made in the supernatant. *Superoxide dismutase (SOD) activity* was determined after the inhibition of cytochrome C reduction rate by superoxide radical at λ = 560 nm [28]. *Catalase (CAT) activity* was mixed with the enzyme extract by adding H_2O_2-phosphate buffer, and the activity was measured at 240 nm using a spectrophotometer and expressed as µmol/min/mg protein [29]. *Glutathione peroxidase (GPx) activity* was determined by oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) at λ = 340 nm in the presence of t-butyl [30]. *Glutathione reductase (GR) activity* was measured using Beutler's method. One enzyme unit is defined as the oxidation of 1 mmol NADPH per min under the assay condition (25 °C, pH 8.0) [31]. *Glucose 6-phosphate dehydrogenase (G6PD) activity* was determined by monitoring NADPH production at 340 nm and 25 °C [31]. *Malondialdehit (MDA) level* is based on the spectrophotometric measurement of the pink-red color formed as a result of the reaction of MDA with thiobarbituric acid [32]. *Reactive Oxygen Species (ROS)* in fish was determined with some modifications of Gupta et al [33]. Tissues from each fish were homogenized separately in Tris-HCl. Homogenate samples in a volume of 100 µL were mixed with 1 mL of the same buffer and 5 µL of 10 µM 2′,7′-dichlorofluorescent diacetate (DCFDA). The resulting mixtures were incubated at 37 °C for 30 min (Lab. Companion SI-600 incubator shaker, Jelio Tech., Gangnam, Korea). After incubation, fluorescence intensity was measured in samples using a fluorescent spectrophotometer LS55 (PerkinElmer, Waltham, MA, USA) with λ = 485 nm excitation and λ = 525 nm emission.

Statistical analyses and results' interpretation: The statistical analysis of the obtained data as a result of the analyses aimed at interpreting the data of MPs, the interaction of fish species and size with the MP presence, and the interaction of MP presence and oxidative stress findings, was determined at the 0.05 level by using the Statistical Analysis SPSS package program (version 20.0). Differences in oxidative stress response of fish between species in each of the provinces were subjected to Duncan's multiple comparison test by performing general linear analysis (GLA).

3. Results

3.1. Average MP, Total MP and Physical Characteristics in Fish Samples

The morphological characteristics and average MP, total MP, mean length, body weight, and GIT weight of 80 fish samples at different sites are shown in Table 1.

Table 1. A summary of the physical properties, gastrointestinal tract weight, total MP and average MP of different fish species in each city.

City	Species (n = 7)	Average Fish Weight (g)	Average Fish Length (cm)	Average Gastrointestinal Tract Weight (g)	Average Number of MP	Total MP
Bingöl	*Atherina mochon*	51.60 ± 17.69	18.97 ± 2.62	1.83 ± 1.27	11.71 ± 4.99	82
	Capoeta umbla	124.76 ± 38.74	22.24 ± 2.32	12.45 ± 2.89	7.00 ± 5.29	49
	Squalius squalus	38.51 ± 8.60	14.71 ± 1.09	2.11 ± 0.32	15.14 ± 5.70	106
	Blicca bjoerkna	39.88 ± 21.28	14.46 ± 2.97	1.08 ± 0.54	13.29 ± 9.88	93
	Garra rufa	19.46 ± 3.30	11.29 ± 1.00	1.30 ± 0.50	13.14 ± 2.41	92
	C. trutta	44.49 ± 13.38	14.81 ± 1.70	5.36 ± 2.86	12.86 ± 1.21	90
Erzincan	*C. umbla*	36.02 ± 35.61	14.24 ± 4.24	3.34 ± 3.68	9.43 ± 2.23	66
	C. trutta	63.86 ± 14.07	16.63 ± 0.45	11.01 ± 4.19	6.00 ± 1.63	42
	Cyprinus carpio	229.45 ± 180.19	24.90 ± 7.42	26.50 ± 21.31	7.86 ± 2.97	55
	B. bjoerkna	63.06 ± 29.11	17.74 ± 2.96	6.21 ± 1.90	8.43 ± 2.64	59
Erzurum	*Cyprinus carpio*	228.82 ± 163.14	24.99 ± 5.11	29.67 ± 24.72	7.57 ± 3.51	53
	Mugil cephalus	213.34 ± 65.62	24.13 ± 2.61	18.42 ± 6.24	6.71 ± 2.69	47

3.2. Abundance of Microplastics in Fish

In the total MP amount in fish species caught from different provinces, six fish species (n = 7, total 42 fish) had a total of 512 MPs in Bingöl, two fish species (n = 7, total 14 fish) had a total of 100 MPs in Erzurum, and four fish species (n = 7, total 28 fish) had a total of 222 MPs in Erzincan.

When examined on a species basis in the Bingöl, Erzurum and Erzincan provinces; among the six different species caught in Bingöl, the maximum MP count was determined in *S. squalus* (20.7%) and *B. bjoerkna* (18.2%), in Erzincan province the maximum MP count was found in *C. umbla* (29.7%) and *B. bjoerkna* (26.6%). In Erzurum province, *C. carpio* (53.0%)'s amounts were found to be higher than the other species (Figure 2).

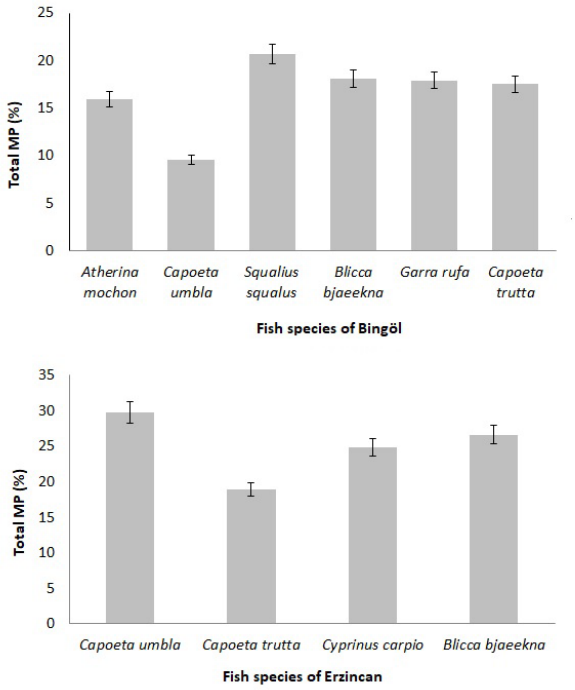


Figure 2. *Cont.*

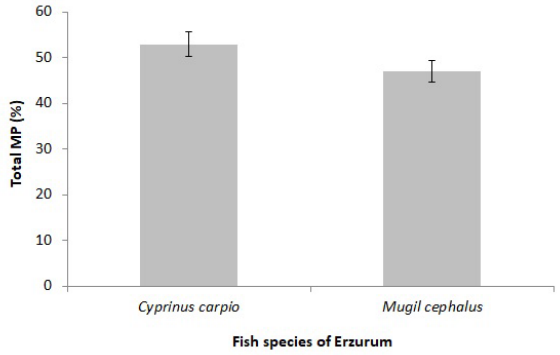


Figure 2. Total microplastic rates in fish species of different regions.

No significant difference was found when the MP presence of the same species in different provinces was compared ($p > 0.05$) (Figure 3).

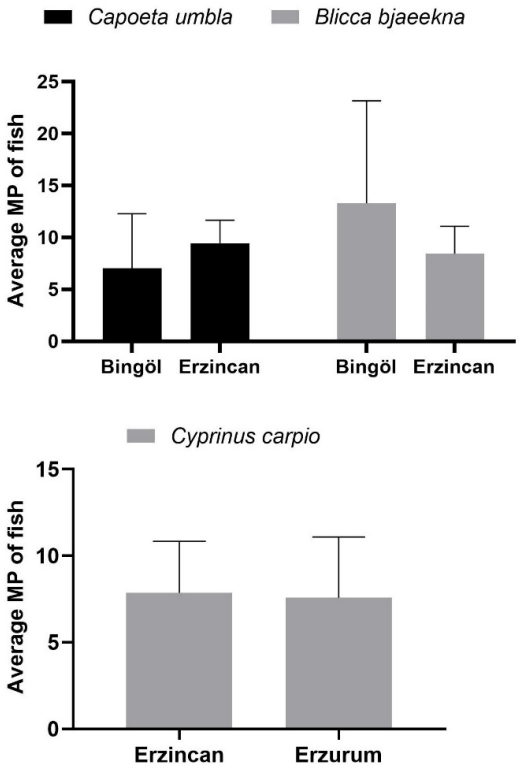


Figure 3. Variation of microplastics in similar fish species in different regions. Data are expressed as means ± S.D. (n = 7; ANOVA with Tukey's post hoc test).

3.3. Physical Characterization of Microplastics

Microplastics in different shapes (fragment, fiber, pellet/bead, and film) were determined in the digestive systems of the examined fish species (Figure 4). Looking at the shape rates by province; in Bingöl, fragment was found in *A. mochon* (52.4%), *C. umbla* (46.9%), *S. squalus* (47.2%), *B. bjoerkna* (51.6%), *G. rufa* (54.3%), and *C. trutta* (45.6%). Fiber was dominant in Erzincan at *C. umbla* (56.1%), *C. trutta* (57.1%), *C. carpio* (49.1%) *Blicca bjoerkna* (44.1%). The same situation was also valid in Eruzurm where fiber MP was found in *C. carpio* (52.8%) and *M. cephalus* (48.9%) (Figure 4). There was no difference between *B. bjoerkna* (Bingöl and Erzincan, fragment) and *C. carpio* (Erzincan and Erzurum, fiber) species taken from different provinces, and a similar MP shape was dominant. However,

the predominant microplastic form was fragment in Bingöl and fiber in Erzincan, in the *C. trutta* species (Figure 5).

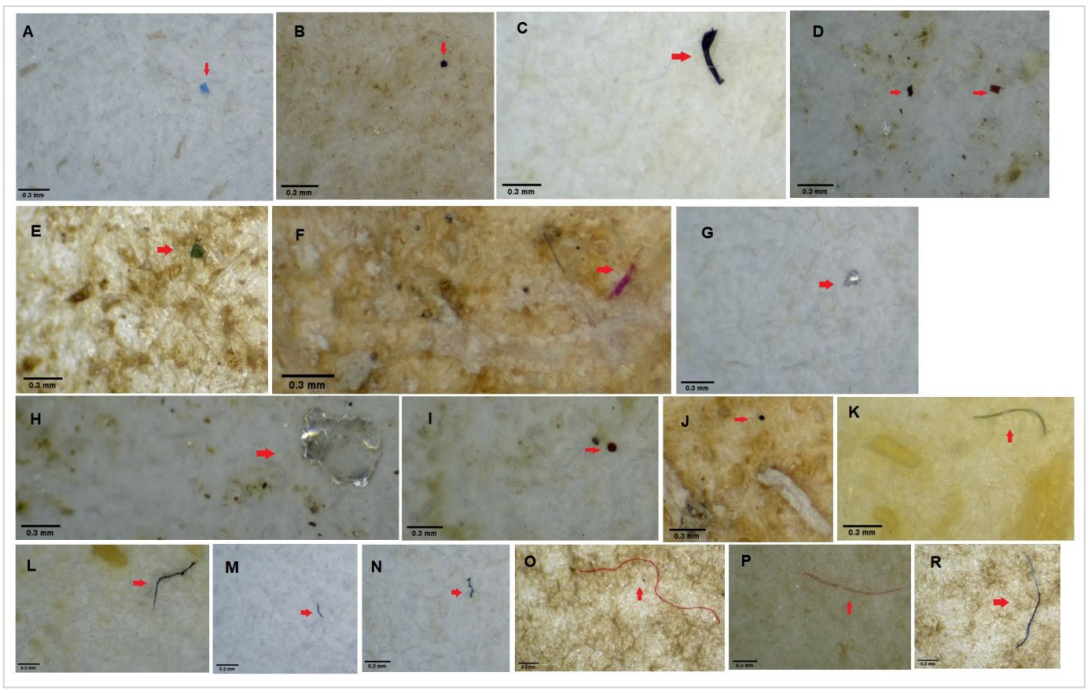


Figure 4. Images of MP found in the gastrointestinal tract of fish (red arrow pointed): fragments (**A**–**F**), films (**G**,**H**), pellets/beads (**I**,**J**), and fibers (**K**–**R**). Scale bars represent 0.3 mm.

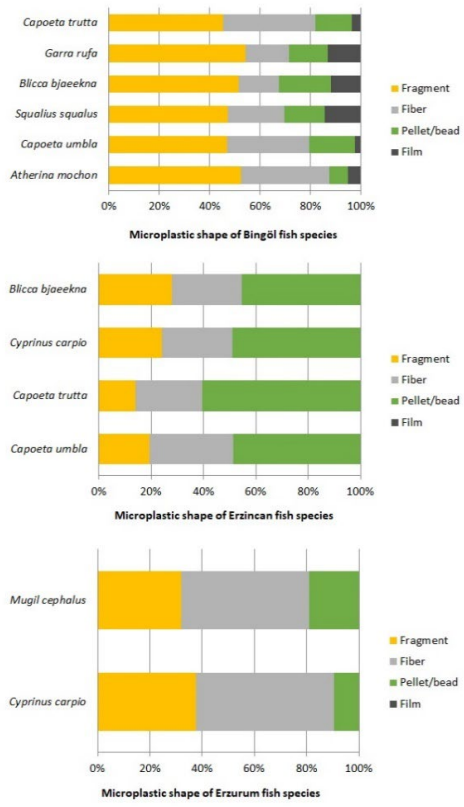


Figure 5. Proportional distributions of MP shape in fish species sampled from Erzurum, Erzincan and Bayburt provinces.

The color of MPs (black, blue, grey/whitish, red/pink, yellow, green) in the digestive systems of fish species in the Bingöl, Erzurum and Erzincan provinces were determined as follows: the dominant color in Bingöl was black and the percentages according to the species were: *A. mochon* (37.8%), *C. umbla* (42.9%), *S. squalus* (37.7%), *B. bjoerkna* (26.9%), *G. rufa* (34.8%), *C. trutta* (57.8%) (Figure 6). In Erzincan, the dominant color was determined as black in all species and the ratios were: *C. umbla* (63.6%), *C. trutta* (83.3%), *C. carpio* (69.1%), *B. bjoerkna* (57.6%) (Figure 5). The dominant MP color was determined as black in Erzurum's fish species in *C. carpio* (66.0%) and in *M. cephalus* (83.0%) (Figure 6). The second most dominant microplastic color was determined as blue in all three provinces (Figure 6). The MP sizes (0–50, 50–100, 100–200, 200–500, 500–1000, 1000–2000, 2000–3000 µm) differed according to the province and fish species. In Bingöl province, the most dominant MP size in the digestive systems of fish was in the range of 50–100 µm with percentages of: *A. mochon* (30.5%), *C. umbla* (40.8%) and *C. trutta* (44.4%). However, in the same province, in the species of *S. squalus*, *B. bjaeekna* and *G. rufa*, the dominant range was 0–50 µm with percentages of 35.8%, 39.8%, and 38.0%, respectively (Figure 5). The dominant microplastic size was determined as 50–100 µm in Erzincan provinces' fish species of *C. umbla* (25.8%), *C. trutta* (45.2), *C. carpio* (45.5%), *B. bjoerkna* (44.1%) (Figure 6). In Erzurum, in the *C. carpio* (39.6%) species the dominant size was in the 50–100 µm range. Another fish species, *Mugil cephalus* (42.6%), showed the dominant microplastic size of 0–50 µm (Figure 6).

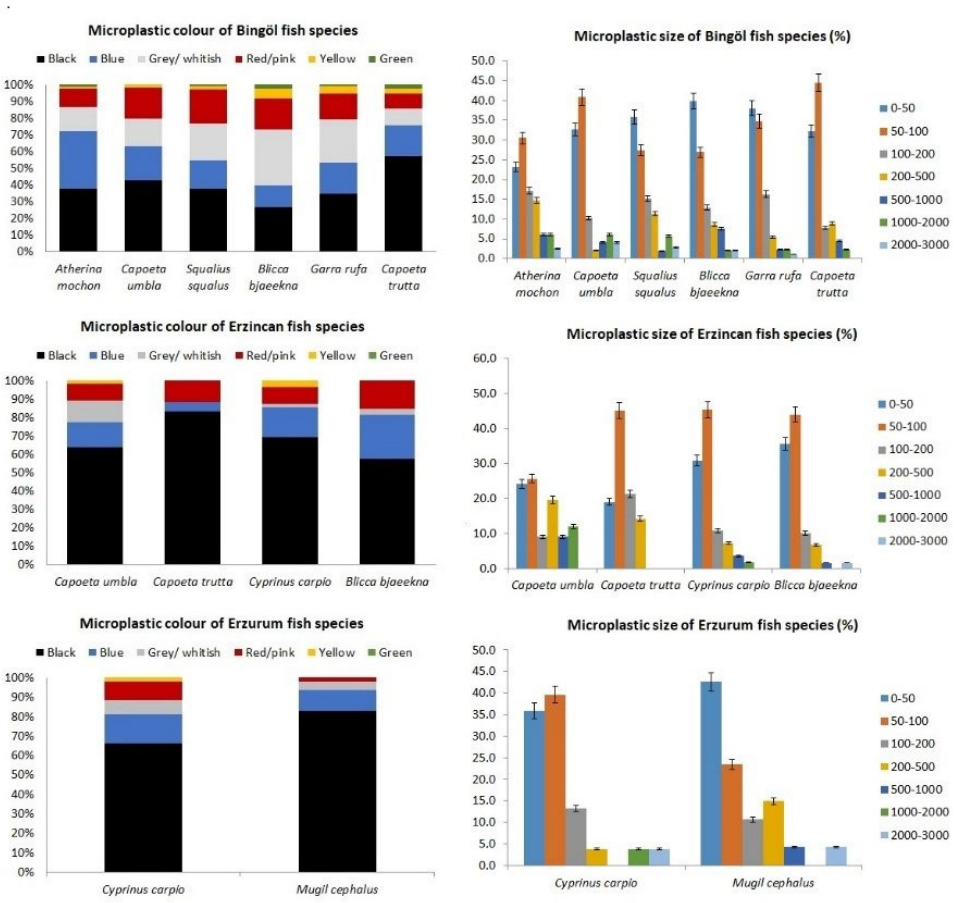


Figure 6. Proportional distributions of MPs based on color and size in fish species sampled from Erzurum, Erzincan and Bayburt provinces.

3.4. Characterization of Microplastics

More than 10% (85 MP) of the total determined MP amount (834 MP) and those larger than 40 µm were evaluated using FTIR spectra. Common polymer types of 85 selected MPs are Polypropylene (PP), Polyester (PET), Polysulfide rubber (PCR), Polychloroprene chlorine (PCP), Polyvinyl chloride (PVC), Polyisobutylene rubber (PIB), Ethylene propylene

(EPR), Ethyl-acrylate (EA), Polyacrylate (PA) and their percentages are given in Table 2 and Figure 7.

Table 2. Polymer composition of ATR-FTIR verified particles.

Polymer Type of Bingöl, Erzincan and Erzurum	Microplastic Percentage (%)
Polypropylene (PP)	31.8
Polyester (PET)	20.0
Polysulfide rubber (PCR)	7.1
Polychloroprene chlorine (PCP)	8.2
Polyvinyl chloride (PVC)	8.2
Polyisobutylene rubber (PIB)	8.2
Ethylene propylene (EPR)	4.7
Ethyl-acrylate (EA)	5.9
Polyacrylate (PA)	5.9

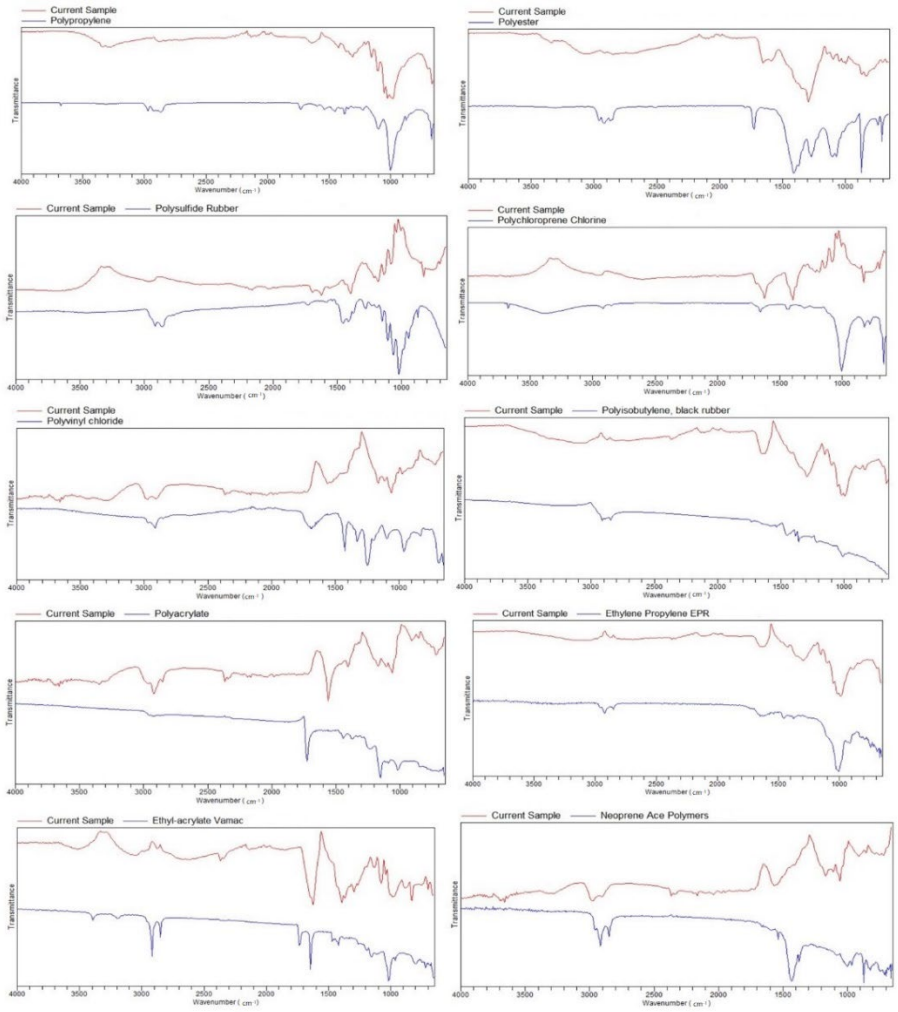


Figure 7. Examples of polymer spectra with ATR-FTIR analyses of observed MPs in fish gastrointestinal tract identified among the 85 microplastics.

3.5. Enzyme Results

In order to determine the level of oxidative stress caused by MPs in the sampling areas, the activities of a number of enzymes in the obtained species were evaluated.

In the analyses made between provinces, the highest SOD and CAT activity was determined in Erzurum. When the GPx, GR and G6PD enzymes were evaluated, the

highest activity was detected in the species caught in Bingöl province. ROS, which is the indicator of oxidative damage in the fish species from the sampling areas, was ranked as the highest in Bingöl > Erzincan > Erzurum, while MDA levels were recorded as Bingöl > Erzurum > Erzincan from high to low. When evaluated in general terms, it can be said that oxidative damage is high in the species sampled in the Bingöl province. The lowest SOD-CAT activities were also obtained in this province (Figure 8).

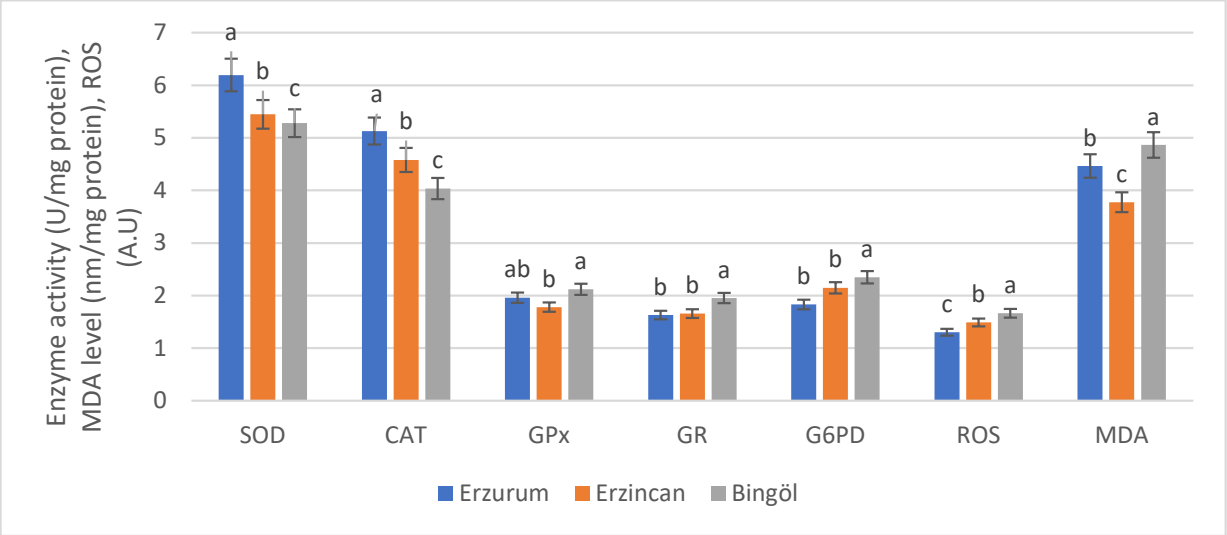


Figure 8. Antioxidant capacity and ROS and MDA content in fish species sampled from Erzurum, Erzincan and Bayburt provinces. Data are presented as mean ± SE. Superscripts (a,b,c) show statistically significant results compared to control ($p < 0.05$).

When the enzyme activations are evaluated in terms of the sampled species, SOD-CAT activity is the highest. The species with the lowest ROS activity was determined as *M. cephalus*. The species with the lowest SOD activity was determined as *Gobius niger*, where GPx and G6PD enzymes were detected in higher amounts compared to other species. *Squalis*, the species with the lowest CAT activity, had a high MDA level (Figures 9–11).

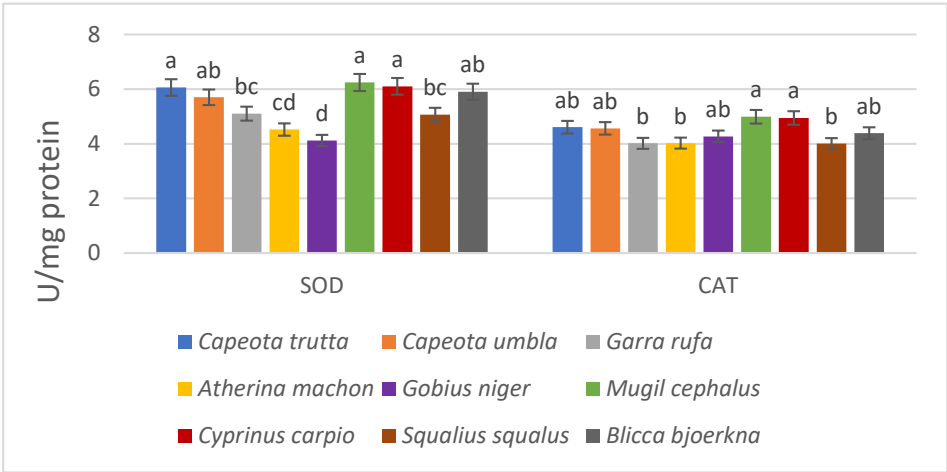


Figure 9. SOD-CAT variation in fish species sampled from Erzurum, Erzincan and Bayburt provinces. Data are presented as mean ± SE. Superscripts (a,b,c) show statistically significant results compared to control ($p < 0.05$).

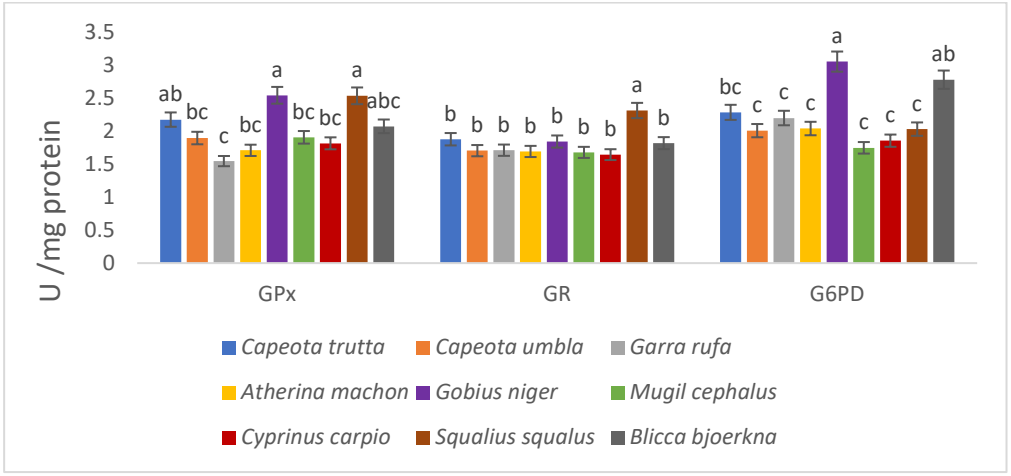


Figure 10. GPx-GR-G6PD variation in fish species sampled from Erzurum, Erzincan and Bayburt provinces. Data are presented as mean ± SE. Superscripts (a,b,c) show statistically significant results compared to control ($p < 0.05$).

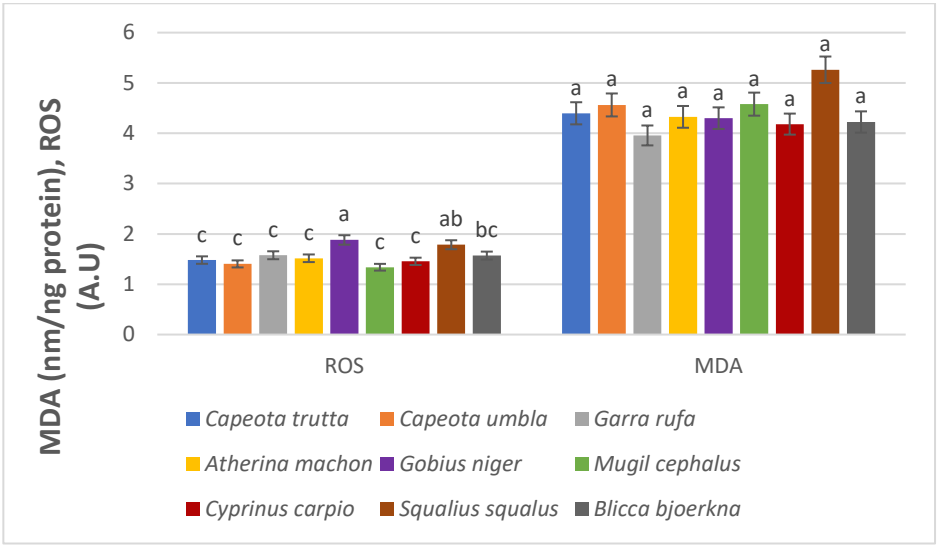


Figure 11. ROS-MDA content variation in fish species sampled from Erzurum, Erzincan and Bayburt provinces. Data are presented as mean ± SE. Superscripts (a,b,c) show statistically significant results compared to control ($p < 0.05$).

4. Discussion

Environmental monitoring agencies around the world still do not have standardized suggestions for sampling methods of MPs in water bodies, making it difficult to compare our results with other studies. In the presented study, the highest total MP was determined in the Bingöl *S. squalis* species. Besides, fragment-shaped microplastics were numerically predominant in all sampling areas [(Bingöl *G. rufa* (54.3%), Erzincan *B. bjoerkna* (44.1%)]. These data are also coherent with data in the literature, where fragment is the most common morphological type [34–37]. Although fibers were also known as a prevailing morphology [38–41], they were recorded as the second most widespread morphology in Turkey. Our findings suggest that the fragments are from secondary origins, possibly as a result of the degradation of freshwater mesoplastics. MPs get into freshwater ecosystems primarily from secondary MPs produced by the impairment of bigger pieces of plastic [5,10,42,43]. Therefore, MP presence in water can be attributed to many sources, including discharge of untreated wastewater, road runoff, infiltration of landfill leachate, effluent from wastewater treatment plants, and inadequate waste disposal. This explanation is provided due to the

absence of industries around the sampled streams that could be primary sources of microplastics. Extensive connections can be made regarding the observed MP morphologies and potential sources. The point in question earlier, that fragments were the most prevalent morphology, suggests that fragmentation of bigger fragments of plastic and garbage could be an important source of MPs from the sample area. Yonkos et al. [44] and Mani et al. [45] reported that higher amounts of MPs can be found closer to urban areas, while particle numbers may be regularly higher near urban areas. Horton et al. [46] reported that, in addition to finding large numbers of particles downstream of urban discharge points, they also found particles in rural areas where human inputs are expected to be low.

Recent research has revealed that fibers mainly arise from the use and washing of clothing, reaching aquatic mediums through the drainage of domestic wastewater [47,48]. Observation of a high fiber content in the sampling areas [(Erzincan *C. trutta* (57.1%), Erzurum *C. carpio* (52.8%))] suggests that wastewater treatment may be poor in these provinces and domestic pollution may be an important factor. Also, one explanation for this investigation is that the fibers can become entangled and form agglomerates, stoppering the organs and thereby preventing them from being excreted from the organism along with the feces [49]. While MP shape and color distributions vary between habitats and trophic groups, the dominant color determined in the three different provinces in the present study was black. In the provinces sampled, the highest values for the dominant color black were determined as: Erzurum *M. cephalus* (83.0%), Bingöl *C. trutta* (57.8%), Erzincan *C. trutta* (83.3%). This data is coherent with the findings of Atamanalp et al. [2]. This may be due to the natural wear of vehicle tires, which are easily washed off the road in rainy weather and can enter the aquatic body through the city's streams and drainage systems [50].

In the natural environment, the large surface area and hydrophobicity of MPs allow them to accumulate hazardous chemicals (for example, heavy metals and hydrophobic organic pollutants) at a significantly higher concentration than in the ambient matrix [26,51]. This concern has sparked increased research interest in the consolidated effects of MPs and related xenobiotics on aquatic living. Plastic contaminants in aquatic mediums decompose into smaller pieces through sunlight, waves, and physical and chemical reactions with aquatic organisms. Substances that decompose to MP level can be mistakenly taken as food by aquatic organisms [52].

Potential MP entry points through direct uptake into aquatic food webs are found at nearly every trophic level. Phytoplankton can directly adsorb nanoplastics through their cell walls, and zooplankton such as Daphnia and fish can take up nanoplastics and MPs directly from the water column [53]. MPs occur at particularly high concentrations in marine and estuarine environments, with the accumulation of various MPs in freshwater and marine fish species [54]. After ingestion, MPs can accumulate in the gastrointestinal tract of fish, causing blockages throughout the digestive tract and reducing feeding by creating a feeling of satiety [55]. MP particles accumulated in the body pass through the cells and enter the circulatory or lymphatic system and are distributed throughout the body. MP intake may cause problems with fish nutrition and growth due to structural and functional disruption of the gastrointestinal tract [56].

The difference in MP accretion by fish is primarily related to ingestion style (feeding strategy including ingestion, sucking and filter feeding [57,58]), intestinal structure, and plastic contamination of habitat [40,59,60]. According to one theoretical model, fish tend to ingest MPs that are more like their prey. Fish food includes aquatic plants, algae, plankton and small organisms, humus and organic/inorganic wastes; since some of the MPs have a similar morphology to these, they create a visual illusion and are effectively taken up by fish [26,51]. In freshwater ecosystems, the roles of habitat and habitat-related feeding choice are less clear and are fairly diversified according to various research [61,62]. In general, benthic organisms with chemosensory control of feeding near the bottom of freshwater habitats are thought to be more exposed to MPs because concentrations in the sediment are conceived to be greater than in the water column [9,10]. Besides, the existing findings suggest that an enhanced sense of taste limits the unintentional ingestion of MP particles.

The fact that *C. trutta* has a large amount of mud in its stomach during the summer months when digestion is fast and that phytoplanktonic organisms that are abundant in the environment are among the nutritional organisms that participate in their gastrointestinal system may be due to the fact that the mouth of the fish is ventral, and it does not have a particular food preference [63]. *G. rufa* feeds on aquatic grasses and plankton and algae on the surfaces of rocks and stones in water [64]. *S. squalus* feeds on a variety of plant and animal materials. Larger individuals are solitary, and the largest feed mainly on fish. Considering the diet of the examined species, the fact that MPs enter via the trophic chain is confirmed. In the area, other uptake pathways, such as transfer via the food chain, may have a more extensive influence on the MP load of benthic fish species [65,66]. In this research, it was determined that the lowest total MP in *C. trutta* species caught from the Erzincan sampling area was 42 per/MP and 57.1% was fiber, the dominant color was black, and the size was 50–100 µm. The highest total MP was recorded in the *S. squalus* species, which was caught from the Bingöl sample area, as 106 units/MP and fragments at the rate of 47.2%, the dominant color was black, and the size was 0–50 µm.

Ingested MPs can lead to a variety of harmful effects on fish. Pure plastics can induce acute inactivity, expenditure of energy reservoirs [67], and liver inflammation, while MPs connected with consistent organic contaminants have been exhibited to provoke chronic or acute toxic influences [53]. The fish liver is the tissue most used to evaluate metabolic disorders caused by MPs in fish species because this organ is primarily responsible for the detoxification and inactivation of exogenous compounds [68].

Considering that oxidative stress arises from the imbalance between the formation of oxidative combination and the performance of antioxidant defense systems, and this defense is caused by the presence of chemical pollutants, it is expected that the enzymatic response will undergo changes as a result of the presence of microplastics.

The exact pattern of oxidative stress induced by MP exposure is difficult to define, but it does affect oxidative homeostasis [69]. In addition, MPs can also adhere to fish skin or migrate to other tissues such as muscle gill and liver [70,71]. It has also been documented that very fine plastic particles can pass from living cells to the circulatory or lymphatic system, causing microplastics to disperse throughout the body [72]. Unfortunately, data on the presence of microplastics in tissues outside the digestive tract of fish are currently very limited.

The production of free radicals and reactive oxygen species (ROS) has a defense system based on the activity of antioxidant enzymes [73]. The antioxidant defense system provides potential biomarkers of stress conditions [74]. Antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD) provide the first line of defense to combat free radical damage. The superoxide radical is typically the first ROS to form, eventually leading to the formation of hydrogen peroxide (H_2O_2) and hydroxyl radicals [75]. When SOD activities were analyzed statistically, the highest activity was determined in *C. trutta*, *M. cephalus* and *C. carpio* species, while the lowest was determined in *G. niger* species. Similar values were found in *C. umbla*/*B. bjoerkna* and *G. rufa*/*S. squalus* species. The highest CAT activity was found in *M. cephalus* and *C. carpio* species, while the lowest was found in *G. rufa, A. machon* and *S. squalus* species. Statistically similar/close results were obtained in *C. trutta, C. umbla, G. niger* and *B. bjoerkna* species. The highest GPx activity was determined in *G. niger* species, while the lowest value was determined in *G. ruffa* species. Similar/closer results were obtained in *C. umbla, A. machon, M. cephalus, C. carpio* species. The species with the highest GR activity was *S. squalus*, and the other species examined gave statistically similar/close results in terms of GR activity. The species with the highest G6PD activity was *G niger*, except for *C. trutta* and *B. bjoerkna*, other species were evaluated as statistically similar. ROS was evaluated as the highest in the *G. niger* species and statistically similar results were obtained in other species except *S. squalis* and *B. bjoerkna*. Although changes were determined in all species examined at the MDA level, statistically similar results emerged.

Although the balance between ROS generation and antioxidant activity is crucial to protect fish from environmental contamination [76], it is difficult to directly see ROS generation in fish tissues [77]. Besides, the observation of certain phenomena, including

lipid peroxidation following ROS production, may confirm the generation of oxidative stress by pollutions [78]. This situation may be related to the importance of maintaining the oxidative balance of the examined tissues, since detoxification processes are usually triggered when multiple oxidative reactions and high free radical formation occur in the liver [79]. In the study findings, high MDA levels were noted with depletion of antioxidant enzymes in the liver. Among the antioxidant enzymes used by organisms to balance oxidative damage as a result of ROS-induced cellular damage are: superoxide dismutase (SOD), which converts O_2 to H_2O_2; catalase (CAT), which reduces H_2O_2 to water; and glutathione peroxidase (GPx), which detoxifies H_2O_2 or organic hydroperoxides into water [80].

The liver has been used to evaluate metabolic disorders caused by MPs as it is the main organ in charge of the inactivation and detoxification of exogenous molecules [81,82]. In this study, the province with the highest CAT and SOD activities was determined as Erzurum > Erzincan > Bingöl. On the other hand, ROS level was recorded as Bingöl > Erzincan > Erzurum, and MDA level as Bingöl > Erzurum > Erzincan. Particularly, exposure of aquatic organisms to MPs can stimulate the generation of ROS, which leads to oxidative injury to the macromolecules of tissues [49,83,84]. Deterioration of redox homeostasis in fish by toxic substances takes place as a result of a quick rise in the original concentration of ROS as energy is directed to synthetize molecules and antioxidant enzymes that increase antioxidant capacity [85].

Fish are defined by a complex antioxidant system that involves mechanisms such as CAT and SOD as well as GPX and G6PD [86]. When the interactions between species were examined, the highest SOD and CAT activity was determined in the *Mugil cephalus* species. Considering the total MP numbers in fish samples, 47 MP was determined in the *M. cephalus* species. In contrast, CAT and SOD activities were found to be low in the *S. squalus* species, where the highest total MP was determined. At the same time, it can be said that the high levels of ROS and MDA in this species induce oxidative stress depending on the presence and density of microplastics, and on the contrary, this reduces antioxidant levels.

In the presented study, the highest MP amount was obtained in the *Squalis* species in Bingöl. When the ROS and MDA results were examined, the highest values were determined in Bingöl province. Exposure of aquatic organisms to MPs induces ROS generation, which stimulates or inhibits antioxidant response. Furthermore, exposure to MPs disrupts (or affects) glutathione and dependent response cycles in fish, which contributes to antioxidant reactions. Thus, exposure of fish to various MPs causes oxidative damage by disrupting the antioxidant balance between antioxidant capacity and ROS generation. Similar to other toxicants, MPs induced ROS generation in fish or inhibited their antioxidant talent, based on their sensitivity to oxidative stress. Aquatic organisms, especially fish, have evolved antioxidant enzyme functions to inhibit oxidative detriment from ROS production caused by exposure to a variety of toxic materials, including MP exposure [87]. This inhibition was likely related to the oxidative stress reply stimulated by MP exposure. MDA level increased with the presence of microplastics, which supports that microplastics induce oxidative stress. The data show that MPs induce lipid peroxidation in a concentration-dependent manner.

5. Conclusions

This study provides evidence of microplastic contamination of the dominant fish species caught in streams in the Erzurum, Erzincan and Bingöl provinces. Besides the MP abundance, lipid oxidative damage and antioxidant enzymes in MP-containing fish were evaluated. These findings point to oxidative damage and lipid peroxidation damage and suggest a relationship between these changes and the contamination of fish with microplastics. Given the crucial role of fish as a source of protein for humans, continued surveys are strongly recommended to indicate the ecotoxicological effects of MPs on fish from an individual to population level and to scientifically evaluate the threats to these species. Although the majority (>90%) of MPs taken up by organisms are fecal, enabling

the discovery of diagnostic biomarkers to overthrow the introduction of MPs into aquatic mediums to protect the aquatic medium can also help encourage the progress and practice of suitable regulations or policies. Consequently, the use of more comprehensive study advances to screen for the effects of environmental conditions may even increase the likelihood of defining the extent of damaging influences.

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