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Fifty Shades of Pollution: Microplastic Color Diversity in *Mytilus galloprovnicialis* (Lamarck, 1819) from the Black Sea, Bulgaria

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Abstract. Microplastics (MPs) are emerging pollutants of global concern, infiltrating marine environments and posing potential risks to aquatic organisms. The Mediterranean mussel (*Mytilus galloprovincialis*), a widely distributed and economically significant bivalve, serves as a reliable bioindicator for assessing MP pollution. In this study, mussels collected from the Bulgarian Black Sea coast were analyzed to determine the presence, abundance, polymer type, shape, and color composition of accumulated MPs in whole soft tissues. MPs of various colors were detected, with transparent and blue particles being the most prevalent, followed by white, black, red, green, and yellow. The observed chromatic diversity indicates multiple pollution sources, including domestic, urban, and maritime activities. These results highlight the extensive contamination of coastal ecosystems and reinforce the value of *M. galloprovincialis* as a sentinel species for monitoring both the quantity and qualitative attributes - such as color - of MPs in marine environments.

Keywords: microplastics, color, mussels.

Introduction

As reported by Plastics Europe (2024), worldwide plastic production continues to escalate - from 370.7 million tonnes in 2018 to nearly 414 million tonnes in 2023, intensifying concerns over plastic pollution in marine environments. In addition, an estimated 4.8–12.7 million tonnes of plastic materials reach the world's oceans every year, contributing significantly to marine pollution (Narwal & Kakakhel, 2025). Microplastics (MPs), defined as plastic particles smaller than 5 mm, are an emerging contaminant posing an increasing threat to the health of aquatic organisms and marine ecosystems (Thacharodi *et al.* 2024). According to Ugwu *et al.* (2021), polyethylene (PE) represents the dominant polymer type among microplastics worldwide (36%), followed by polypropylene (PP, 21%) and polyvinyl chloride (PVC, 12%). Other common polymers, such as polyethylene terephthalate (PET), polyurethane (PUR), and polystyrene (PS), contribute less than 10% each. It has been proven that Türkiye, Russia, and Bulgaria are the countries that emit the most plastic in the Black Sea (Savuca *et al.* 2022). Furthermore, plastics constitute more than 80% of marine litter across different environmental compartments of the Black Sea, including the seabed, surface waters, and coastal beaches (Aytan *et al.* 2020). As a result, MPs have been widely detected in surface waters, sediments, and marine organisms throughout the region (Aydin et al., 2023). *M. galloprovincialis,* which is found in the Black Sea, is widely regarded as a valuable sedentary marine invertebrate model due to its filter-feeding habits and its ability to bioaccumulate significant amounts of pollutants, including MPs (Belivermiş *et al.* 2016). Additionally, this species is heavily consumed worldwide, serving as an important source of nutrition for the growing global population (Lopez *et al.* 2023; Ozuni *et al.* 2024).

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The primary goal of this study is to investigate the occurrence and characteristics of MPs in *M. galloprovincialis* collected from the Bulgarian Black Sea coast. Specifically, the study aims to quantify MPs' abundance and to characterize particles in terms of polymer type, shape, and color, providing insights into the extent of contamination, potential sources, and the suitability of the Mediterranean mussel as a sentinel species for monitoring MP pollution in this regional marine ecosystem. Here we present the results on the various colors of MPs.

Materials and Methods

Around 5 kg of mussels from the same age-size group (weight 6±2.5 g; length 5.5±1.5 cm) were purchased from a local farm located in the town of Sozopol, Bulgaria, in the summer of 2025. Careful dissection with the use of no plastic equipment was performed, and the soft tissues of 4 individuals were pooled until reaching approximately 20 g. Five samples were frozen and sent in an ice box to an external accredited laboratory, where MP extraction and MP analysis with Agilent 8700 LDIR (Agilent Solutions, Inc., USA) imaging system followed. The mussels were subjected to preliminary treatment to achieve oxidative degradation of organic matter using a solution of KOH (10%) and H_2O_2 (15%), similarly to our previous study (Arnaudova *et al.* 2025). All samples and solutions were handled using metal or glass equipment to minimize plastic contamination. Procedural blanks were included in each batch of samples, and no MPs were detected in the blanks. Laboratory surfaces and equipment were thoroughly cleaned, and all sample processing was conducted under a laminar flow hood to reduce airborne contamination. The particle size range, which was monitored, was classified into 5 categories as previously described (Yancheva *et al.* 2025): < 50 µm, 51–100 µm, 101–300 µm, 301–1000 µm, and 1001-5000 µm. Due to size constraints, only the colors of the largest MP particles could be determined with the Leica DM 2000 LED microscope (Germany); these observations are addressed further below. All MP abundances were expressed as the number of particles per unit mass of dry weight (items/kg d.w.). The Excel program (Microsoft, USA) was used for the MP quantitative analyses.

Results and Discussion

MPs were detected in all analysed *M. galloprovincialis* samples collected from the Bulgarian Black Sea coast. The number of particles per kilogram of soft tissue varied among the five samples, ranging from 38.684 to 800.874 particles kg^{-1}. The individual counts were 106.971, 800.874, 244.498, 450.663, and 38.684 particles kg^{-1}, resulting in an average abundance of 284.338 ± 281,751 particles kg^{-1} (mean ± standard deviation). Out of a total of 1421690 MP particles identified in 5 *M. galloprovincialis* samples, only 17 particles were classified in the larger size range of 1001–5000 µm. Among these larger particles, 6 were transparent, 4 were blue, 2 were white, 2 were black, and 1 each was red, green, and yellow. These findings indicate that while the majority of MPs in mussel tissues are small (<1000 µm), the larger particles exhibit a variety of colors, reflecting multiple potential sources of contamination and highlighting the importance of color as a parameter in environmental MP assessment. Regarding colour distribution, our larger-size class (1001-5000 µm) breakdown (transparent 35 %, blue 24 %, white 12 %, black 12 %, and red/green/yellow each ~6 %) mirrors the dominance of transparent and blue/black particles documented in other studies. According to Rezania *et al.* (2018) and Ugwu *et al.* (2021), the predominant color in marine biota is blue (28.12% fish, 50% marine mammals, 75% turtles), followed by black. However, according to Ugwu *et al.* (2021), in invertebrates, the predominant color is light green. The color of MPs is not only a visually distinguishable feature, but also a key factor influencing their environmental persistence, degradation, and potential toxicity to marine organisms. Color can help trace the origin of MPs (e.g., black MPs often come from industrial products, blue from fishing gear, red/yellow from packaging). Some colors degrade faster than others due to UV exposure or photochemical reactions, influencing persistence in the environment. Colored plastics typically contain pigments or dyes to achieve the desired hue, ranging from organic dyes to inorganic compounds, including heavy-metal-based pigments, such as cadmium (Cd) in reds or lead (Pb) in yellows. Dark-colored plastics, especially black MPs containing carbon black, are particularly resistant to degradation, but may absorb more ultraviolet radiation, which can accelerate photodegradation and facilitate the release of embedded additives. In mussels, which are filter-feeding organisms and bioindicators of environmental contamination, ingestion of colored MPs may therefore introduce both the plastic particles themselves and associated chemical substances. Leaching of pigments, plasticizers, and other additives from these MPs can lead to the accumulation of toxic compounds within mussel tissues, potentially inducing histopathological lesions, oxidative stress, altering enzymatic activity, impairing immune responses, and affecting overall physiological performance. Furthermore, the color of MPs may influence ingestion rates, as certain colors are more likely to be mistaken for food by fish, marine mammals, etc., thereby increasing exposure to both physical and chemical hazards.

Given that humans widely consume mussels, understanding the relationship between MP color, leaching of toxic substances, and biological effects is essential for assessing ecological risks and potential implications for food safety. Consequently, integrating MP color analysis with polymer identification and toxicological assessment provides a more comprehensive understanding of MP pollution in marine ecosystems.

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