

Bioaccumulation of microplastics in tomato plants (Solanum lycopersicum L.) - a pot experiment

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Abstract. Nowadays it is known that microplastics penetrate seeds, roots, stems, leaves, fruits and plant cells, and the ability for translocation and bioaccumulation depends mainly on their size and type. The aim of this study is to explore the bioaccumulation and translocation of fluorescent microplastics in tomato (*Solanum lycopersicum* L.). The biotesting was carried out under controlled conditions, using pots with a volume of 0.1 liters. Each pot was filled to 2/3 of the volume with standardized soil, to which the selected fractions of fluorescent polystyrene microplastics (1 µm, 5 µm, 100 µm) were added at a concentration of 0.01 % v/w. Two seeds were placed in each pot and a 1.5 cm soil layer was added on top. After a period of 40 days, the penetration and accumulation of microplastics in all underground and aboveground organs of tomato (roots, stem, leaves) was observed. The amount of microplastics was higher in the underground biomass (root system) compared to the aboveground biomass (stem and leaves) in all experimental variants. Microplastics accumulated in tomato plants exerted an impact on their growth and biomass synthesis, which is most pronounced in the finest of the studied fractions - 1 µm (p<0.05). Microplastics also had a suppressive effect on the biosynthesis of photosynthetic pigments, and hence - on the intensity of the photosynthesis process, again most pronounced in the fraction of 1 µm (p<0.05). It has been confirmed that the size of plastic particles is an important factor determining their ability to bioaccumulate and the degree of impact on the vital processes of plants.

Key words: fluorescent microplastics, soil pollution, bioaccumulation, food safety.

Introduction

In recent years, there has been an increasing number of studies on the mechanisms of uptake and translocation of microplastics (MPs) in plants. MPs have been shown to penetrate seeds, roots, stems, leaves, fruits and plant cells, but only to a certain extent and depending on their size and species (Jia et al., 2023). It is generally accepted that plants are unlikely to be able to absorb MPs

due to their high molecular weight and large size, which would prevent them from penetrating the cellulose-rich plant cell walls. However, their bioaccumulation in plant tissues has been reported by many researchers (Zhang et al., 2020, 2021; Monkul & Özhan, 2021; Nikolov et al., 2023).

In a recent study Fajardo et al. (2022) used three types of MPs (white, blue and fluorescent blue) within a pot experiment with *Zea mays*

seeds. They found that MPs not only accumulate in plant tissues but also act as pollutants carriers which poses a serious threat to both soil biota and terrestrial plants.

Qi et al. (2018) studied the effects of low-density polyethylene (LDPE) and biodegradable starch-based microplastic films of different sizes on wheat grown in pots. Microplastic films were reported to significantly affect wheat growth, both at the vegetative and reproductive stages. Additionally, biodegradable plastic mulch films had a greater impact on wheat development than polyethylene. Another study by the same team reported that six different types of MPs adversely affected plant biomass, tissue element content, root characteristics, and soil microbial activities in the species *Allium fistulosum*.

Experimental exposure of rice to polystyrene nanoplastics (20 nm) has shown significant distribution of nanoplastics in the intercellular spaces of roots (Zang et al., 2020, 2021). Furthermore, nanopolystyrene (50 nm) was detected in various compartments of primary onion roots after exposure for 72 hours, reflecting that nano-polystyrene is able to penetrate various biological barriers and eventually enter root cells. Similarly, nano-polystyrene (0.2 μm) has been found to be absorbed by the roots of lettuce and wheat, and after that transported into the aboveground organs of plants (Azeem et al., 2021). Thus, due to changes in cell membranes and biochemistry, accumulating nanoplastics have had a detrimental effect on the development of these crops. Therefore, to ensure safe food production, further studies on the interactions of micro- and nanoplastics, as well as their behavior in agroecosystems, are needed.

The aim of the present study is to explore the bioaccumulation and translocation of fluorescent MPs in tomato plants (*Solanum lycopersicum* L.) at laboratory conditions.

Materials and methods

Seed, soil and MPs material

Tomato seeds were obtained from a commercial seed producer GBM Commerce LTD (Lac-tofol, Bulgaria). Seed germination was pre-tested and 100% germination was demonstrated (20 germinated out of 20 seeds planted).

Standardized soil (substrate), purchased from a certified manufacturer (Agro CS, Czech Republic). Substrate characteristics are as follows: total N

content 50-300 mg/l; P content 80-300 mg/l; K content 80-400 mg/l; pH = 5-6.5; Soil organic matter - min 35%.

Polystyrene microplastic spheres (PS MPs) of three sizes have been used, colored with a different fluorescent pigment as follows: 1 μm - yellow; 5 μm - red; 100 μm - blue. The MPs were purchased from Sigma Aldrich (Belgium), and have all the necessary quality certificates.

Bioassay design

Bioassay was carried out in pots with a volume of 0.1 liter. Each pot was filled up to 2/3 of the volume with standardized soil to which one of the studied fractions of MPs was added at a concentration of 0.01% v/w. Two tomato seeds were placed in each pot and a 1.5 cm layer of soil was added on top (Nikolov & Petrova, 2023). Controls were made by a similar manner without adding MPs. All experimental variants were of 4 replicates. Pots were placed in a greenhouse under controlled conditions - air temperature in the range of 22-28°C $\pm$ 3°C and relative air humidity in the range of 45-65% $\pm$ 10%, for a period of 42 days (07.04. -17.05.2025).

MPs uptake and effect assessment

Shoot and root lengths and biomass (analytical balance KERN, AMB-BA-def-1636, Germany) were determined at the end of exposure (42-th day). Intensity of photosynthesis and transpiration, as well as the photosynthetic pigments content in plants, were measured following standard procedures (Petrova et al., 2017). Photosynthesis intensity, transpiration and stomatal conductance measurements were performed with a Q-box CO650 portable photosynthetic system (Qubit Systems Inc., Canada) on three fully developed, undamaged leaves of the same physiological age, with at least 30 values taken for each individual leaf (Petrova & Petkova, 2023).

To confirm the uptake of MPs, the harvested plants were dissected and analyzed using a Axio Scope.A1 fluorescence microscope (Carl Zeiss). Pictures were taken and processed with a digital camera adapted to the microscope - ProgRes CapturePro.

Statistical analysis

Descriptive statistics (min, max, mean, median, mode, SD) was applied and the relationships

between the studied parameters of tomato plants and MPs were tested using Spearman rank correlation coefficients. All analyzes were performed using SPSS 20.0 software (IBM SPSS Statistics, Armonk, NY, USA).

Results and Discussion

The bioassay assessing the impact of MPs on tomato plants started on 07.04.2025, and the first germination of seeds was observed on the 3rd day in the pot with 5 μ m MPs (Fig. 1A). On the 14th day (20.04.2025) all seeds planted in the controls and in the experimental variants had already germinated (100% germination) (Fig. 1B), that is, no impact of MPs on the germination process of tomato was recorded. These results are to some extent in con-

tradition with the data of Liao et al. (2019), who found that polystyrene with dimensions of 100 nm had a strong inhibitory effect on tomato seed germination, and polystyrene with dimensions of 5 μ m had a medium inhibitory effect.

During the vegetation period, plant growth was monitored (Fig. 1C – 1H), and the length of the above-ground organs was periodically measured. Data from biometric measurements on the length of shoot growth (cm) allow us to objectively assess the differences in the initial stages of the development of the test object depending on the size of the applied MPs. Statistically, more intensive growth was found in the experimental variants with added microplastics of the 1 μ m and 5 μ m fractions ($p < 0.05$).

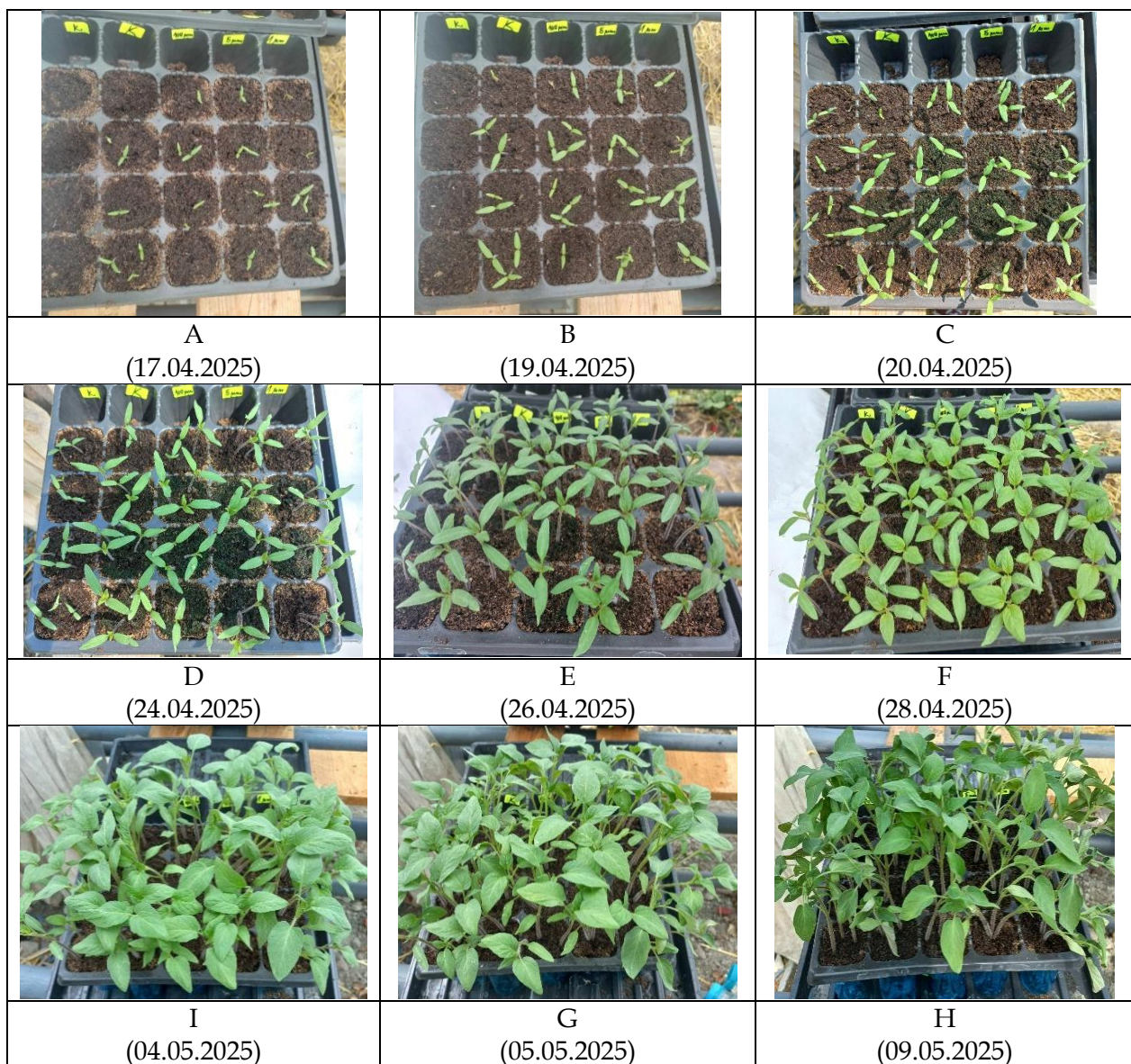


Fig. 1. Photos of the different phases of tomato plants development under MPs impact.

The results of the measurements of the biometric indicators at the end of the experiment (42th day) showed that the length of the above-ground part of the studied tomato plants varied significantly, taking values from 21.0 cm (1 μ m MPs) to 30.5 cm (5 μ m MPs). The differences in shoot length of the experimental and control plants were significant only in the variant with 1 μ m MPs ($p < 0.05$). Regarding the root length, values from 5.5 cm (control) to 21.3 cm (5 μ m MPs) were measured, and statistically significant differences were found for 5 μ m MPs ($p < 0.05$) (Fig. 2A).

The shoot biomass of the studied tomatoes ranged from 1.171 g (5 μ m MPs) to 2.616 g (control), and root biomass ranged from 0.244 g (1 μ m MPs) to 0.771 g (100 μ m MPs) (Fig. 2B). Statistical analysis showed the existence of significant differences only between the variant with 1 μ m MPs and the control ($p < 0.05$). The results obtained confirm that the seed germination stage is a less sensitive stage in ontogenesis compared to the growth stage, which could be used to determine the impact of MPs on physiological processes in plants.

The process of photosynthesis is also well known for its high sensitivity to changes in the plant organism, therefore it is also widely used as a biomarker for stress conditions (Petrova et al., 2019). Another frequently used biomarker is the amount of photosynthetic pigments in the leaves, as well as the ratios between the different functional groups of pigments (Petrova et al., 2017).

As a result of the physiological analyses, it was found that MPs of two lower fractions (1 μ m and 5 μ m MPs) exerted a suppressive effect on the biosynthesis of chlorophyll and carotenoids in tomato plants ($p < 0.05$), while for the 100 μ m MPs the effect was stimulatory ($p < 0.05$). The ratio between green and orange pigments in the control and experimental plants was significant for all studied MPs fractions ($p < 0.05$) (Fig. 3).

The photosynthesis process was significantly inhibited in all test plants, being most pronounced in the variants treated with 100 μ m MPs ($p < 0.05$) (Fig. 4A). Regarding the intensity of transpiration, lower values were reported in all test plants compared to those measured in the control, which are not statistically reliable (Fig. 4B).

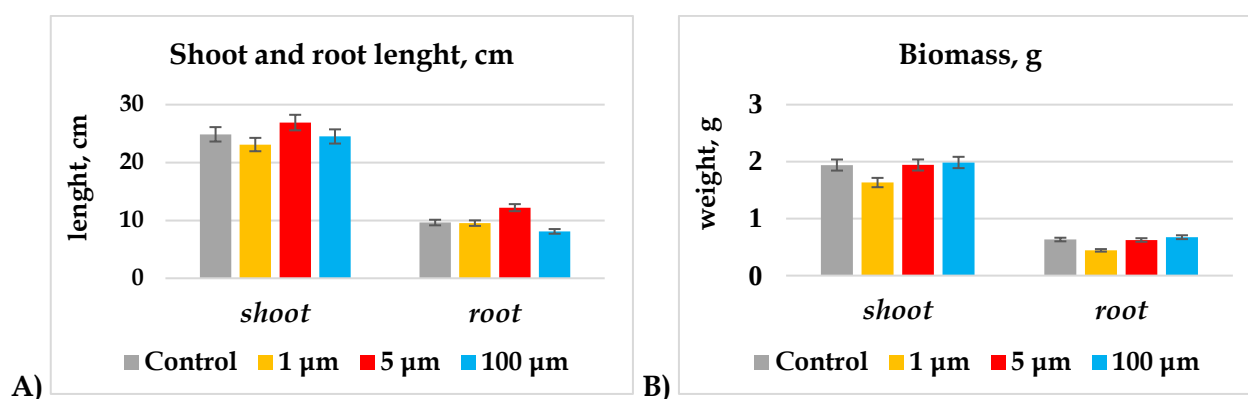


Fig. 2. Results from the biometric measurements of tomato plants under MPs exposure.

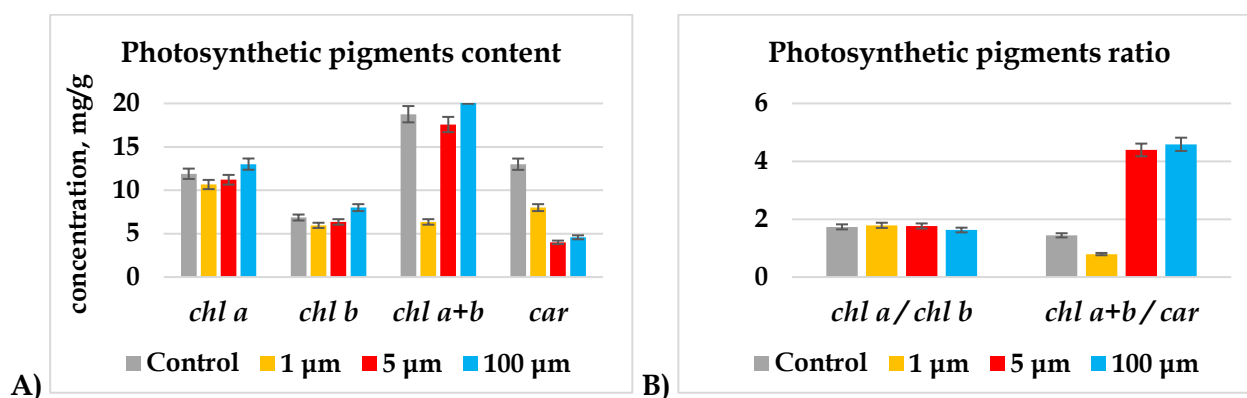


Fig. 3. Effect of MPs exposure on pigment content in tomato leaves.

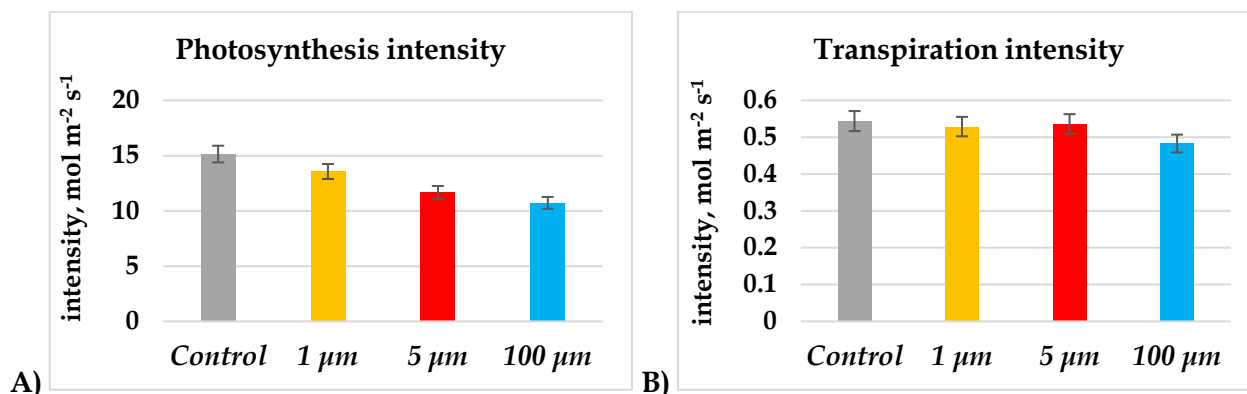


Fig. 4. Effect of MPs exposure on physiology of tomato plants.

The presence of fluorescent microplastics (1 µm, 5 µm, 100 µm) was found in all organs of the tomato plants. The highest particle number was observed in plants treated with the finest fraction 1 µm MPs. Generally, the number of MPs was higher in the underground biomass (root system) compared to the above-ground biomass (stem and leaves) in all experimental variants (Fig. 5).

Based on the literature review, it could be noted that the presence of microplastic particles in plant tissues and organs probably led to the observed alterations in photosynthesis, chlorophyll biosynthesis and tomato physiology as a whole. This had an impact on the growth and development of cultivated plants, as well as on the synthesis of biomass.

The impact of MP on photosynthesis in maize was expressed by changing the photosynthetic rate (positive), transpiration rate (no effect), stomatal conductance (no effect), intercellular CO₂ concentration (negative) (Lian et al., 2021). Another study on maize revealed that PVC MPs (75-220 nm, 0.02 g/150 g soil) had no significant effect on the photosynthetic quantum yield of photosystem-II Y(II), maximum quantum yield (FV / FM) and FV / F0, while on the maximum electron transport rate (ETR) they had a suppressive effect (Pehlivan & Gedik, 2021).

Similar results were reported by Dong et al. (2021), who found a decrease in chlorophyll content, photosynthesis rate and transpiration in red lettuce under the influence of polystyrene microplastic granules. Li et al. (2020) also found a significant decrease in the length and biomass of the underground and aboveground parts of cucumber under the influence of polystyrene nanoplastic

granules (fractions 100 nm, 300 nm, 500 nm, 700 nm). This is also confirmed by the observed changes in the intensity of photosynthesis and the decrease in the amount of photosynthetic pigments. Another studied species included watercress, for which the lack of effect on germination and a stimulating effect on the growth and accumulation of biomass in the aboveground part of plants, induced by different types of MPs, has been proven (Pignatelli et al., 2020).

Tomato plants significantly increased their biomass when microplastics (PP and PET) were added to the soil, but crop yield decreased (Hernandez-Arenas et al., 2021). Overall, low-density plastics did not affect the biomass of the bean cultivars studied, although inhibition was found below the toxicity of biodegradable MP/NP (Meng et al., 2021).

A reduction in both fresh and dry biomass of *Cucurbita pepo* was observed under the influence of PE, PVC and PET at 0.1-0.2% for 28 days (Colzi et al., 2022). These findings confirm that plants respond to MPs/NPs in a species- or cultivar-specific manner.

Studies have shown that smaller MPs/NPs tend to penetrate plant tissues more easily, potentially leading to greater oxidative stress compared to larger MPs/NPs. When MPs are degraded to nanosized particles, their surface-to-mass ratio increases and enables the particles to penetrate directly through lipid membranes (de Souza Machado et al., 2019).

This fact confirms the need to conduct more detailed studies in laboratory and field conditions to track all possible effects of MPs during the full vegetative development cycle of tomatoes.

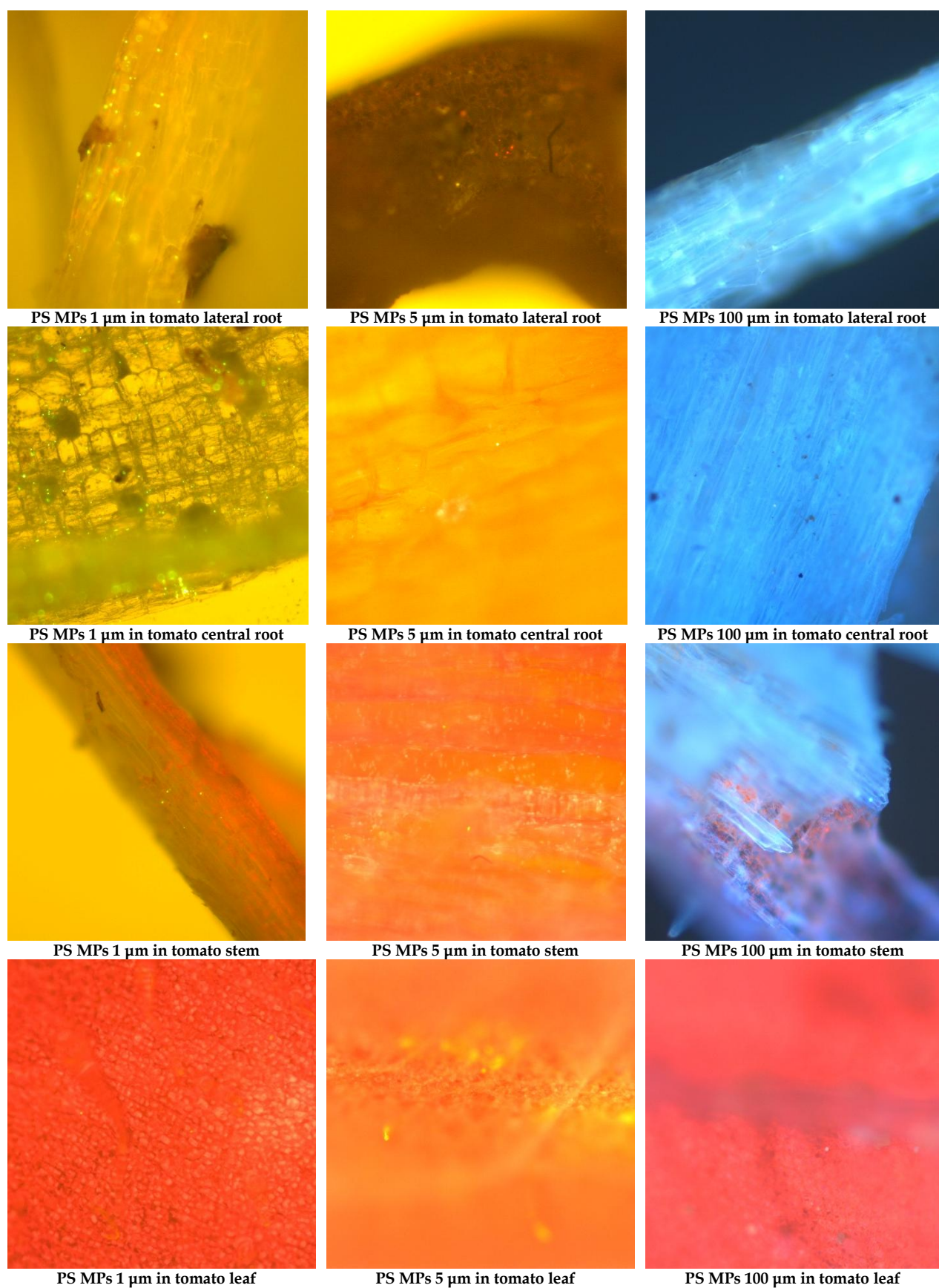


Fig. 5. Microscopic observation of fluorescent MPs in all parts of treated tomato plants.

Conclusions

The biotest with fluorescent MPs demonstrated that the studied polystyrene fractions do not affect the germination of seeds from test tomato plants. The penetration and accumulation of MPs from the three studied fractions (1 µm, 5 µm, 100 µm) in all organs of tomato plants (roots, stem, leaves) has been proven. Furthermore, accumulated MPs in plant tissues exerted an impact on growth and biomass synthesis, most pronounced at the finest of the studied fractions - 1 µm polystyrene beads ($p < 0.05$). MPs also have a suppressive effect on the photosynthetic pigments content, and hence - on the intensity of the photosynthesis process, most pronounced in the 1 µm fraction also ($p < 0.05$). Therefore, the size of MPs is an important factor determining their ability to bioaccumulate and the degree of impact on the vital processes of plants.

The present study demonstrates that MPs in the soil are rapidly absorbed by plants through the root system, transported even to the most distant above-ground organs and accumulated in them. Thus, they have a significant impact on plants, which in above-ground organs manifests itself as the suppression of one of the fundamental processes - photosynthesis, responsible for the synthesis of the necessary compounds for their existence. Future comprehensive studies are needed to more fully reveal the impact of microplastics on plants and their ability to be transmitted through food chains, reaching humans. Addressing this problem has not only fundamental scientific importance, but also a real practical aspect, concerning both the safety of plant production obtained from agricultural lands contaminated with plastics, the safety of animal production obtained from animals fed contaminated feed, and the potential risk to human health and food security.

Acknowledgments

This research was funded by Bulgarian National Scientific Fund, grant number KP 06 N 86/12 "Plastics in agricultural lands and main agricultural crops - assessment of the potential risk to food security".

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Received: 20.03.2026

Accepted: 20.06.2026