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A Combined Effect of Mixed Multi-Microplastic Types on Growth and Yield of Tomato

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Abstract: Microplastics (MPs) are plastic particles ranging from 1000 to 5000 µm in diameter, posing a growing environmental and health risk. Composting is an excellent way to add nutrient-rich humus to the soil to boost plant development, but it also pollutes agricultural soil with MPs. Previous research has shown that MPs can threaten plant development, production, and quality, hence they must be studied. This study examined how a mixture of three MP types—polyethene (PE), polystyrene (PS), and polypropene (PP)—affected greenhouse tomato plant development. MP types were spiked at 1% *w*/*w* (MPs/soil) in tomato pots, whereas non-spiked growth medium was the control. Statistical analysis was conducted using an analysis of variance (ANOVA) and Tukey's test (95% confidence) to compare treatments and controls. Soil spiked with MPs increased chlorophyll content (SPAD), transpiration rate, photosynthetic rate, and stomata conductance by 5.16%, 16.71%, 25.81%, and 20.75%, respectively, compared to the control but decreased sub-stomata CO_2 concentration by 3.23%. However, MPs did not significantly affect tomato plant morpho-physiological features ($p > 0.05$). Biochemical analysis of tomato fruits showed significant ($p < 0.05$) reduction effects of MPs on carotenoid, total flavonoid, and sugar but increased protein, ascorbate, and peroxidase activity. However, there was no significant difference ($p > 0.05$) in the effects of the combined MPs on total phenolic content. These data imply that whereas MPs did not influence tomato plant physiological and morphological properties, tomato fruit biochemistry was reduced. This raise concerns that an increase in MPs in soils may reduce antioxidant content and negatively affect human health contributing to a decrease in food security.

Keywords: microplastics; vegetable production; polyethylene; polypropylene; polystyrene; *Solanum lycopersicum*

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1. Introduction

Recent studies on emerging pollutants highlight the critical need to investigate compost, a common soil enhancer and carrier of microplastics (MPs) in the soil. MPs are defined as plastic particles ranging from 1 mm to 5 mm in diameter and are ubiquitous in ecosystems [1]. They can act as vectors for contaminants [2,3], contribute to soil pollution, and cause damage to soil structure and fauna [4]. MPs can also affect plant health by blocking root cells and nutrient flow [5–7], particularly when absorbed by stems and leaves [8]. In addition, recent studies have highlighted the phytotoxicity of MPs on various plants, including mung beans (*Vigna radiata* L.) [9], broad beans (*Vicia faba* L.) [10], rice (*Oryza sativa*

L.) [11], corn (*Zea mays* L.) [12], lettuce (*Lactuca sativa*) [13], Garden cress (*Lepidium sativum* L.) [14], wheat (*Triticum aestivum* L.) [15], and tomato (*Lycopersicum esculentum* L.) [16].

MPs in soil can negatively affect plant development and food production by interfering with nutrient absorption and possibly acting as vectors of other contaminants [3] because their abundance in agricultural lands can be linked to municipal solid waste (MSW) compost. Compost is increasingly recognized as a significant route for introducing MPs into soil ecosystems [17,18]. During the composting process, plastic debris from various sources, including household waste and agricultural plastics, is broken down into MPs [19]. These MP-contaminated composts are subsequently spread onto agricultural fields and gardens, facilitating their entry into agricultural soil environments [20]. This pathway does not only introduce MPs directly into the soil but also affects soil properties and microbial communities, potentially altering nutrient cycles and soil health [21]. Understanding the extent and impact of MPs in compost is essential for developing strategies to mitigate their spread and protect soil ecosystems [22].

Tomato is a vegetable crop belonging to the Solanaceae family and is globally recognized as one of the most extensively cultivated fruits [23]. Its versatility and nutritional benefits have led to a continuous increase in tomato production [24,25]. Recently, the pervasive presence of MPs in agricultural soils, including those introduced through compost, has raised concerns about their potential phytotoxic effects on crops. MPs can influence plant growth and development by altering soil properties, disrupting root functionality, and interfering with water and nutrient uptake [26,27].

In plants, including tomatoes, these impacts can manifest as changes in physiological traits such as photosynthetic efficiency, water use, and enzymatic activities, as well as morphological characteristics like root structure and leaf development. Additionally, MPs may alter fruit phytochemicals by affecting the synthesis of key metabolites such as sugars, proteins, and secondary metabolites, which are critical for fruit quality and yield. Despite the growing interest in the environmental fate of MPs, studies on their specific effects on the physiological, morphological, and biochemical parameters of tomato plants are scarce. Also, previous research has predominantly focused on the uptake and effects of polystyrene (PS) MPs in crops such as mung bean [9], broad bean [10], rice [7,11,28,29], corn [12], lettuce [30], and wheat [15,31,32], potentially overlooking the influence of other plastic types commonly found in soil and compost.

In soil environments, polyethene (PE) and polypropene (PP) are some of the most prevalent [33], warranting further investigation into the phytotoxic effects of these plastic types. Notably, the World Health Organization (WHO) categorized PE as carcinogenic in 2017 [34]. A significant portion of plastic waste in MSW consists of PP, PS, and PE [35], and this was confirmed in an unpublished study conducted by the authors on MP contamination in MSW composting. The soil environment, especially after the application of compost, usually contains a combination of MPs from various plastic types, including PE, PS, and PP [18,36]. While studies suggest that plant roots can uptake MPs, the translocation to plant stems and tissues may not be consistent and may depend on factors such as plastic-type, size, and exposure duration [37].

The MP size (1 mm–5 mm) in this study was chosen to investigate other potential influences of MP contamination on plants, excluding uptake and translocation. The concentration of MP (1% w/w) in dry weight of soil and compost will mimic the environmental scenario of heavily contaminated agricultural fields since MP of varying types up to 5% w/w have been reported in MSW compost [38–40]. Also, environmental contamination studies usually consider 1% as the contamination rate to have an insight into advancing or reducing the contamination rate. Additionally, there is an emerging alternative of bioplas-

tics to conventional plastic products. Since fragmentation is easier with bioplastics, MP contamination in agricultural systems may increase in future.

Due to their prevalence, this study investigated the impact of combining three common MP types in compost as contaminants, and their effects on tomato plants, focusing on key physiological, morphological, and biochemical traits to elucidate their phytotoxic implications. The interactions between compost, soil, and MPs, and their influence on the growth and development of tomato plants were explored. This will allow scientists to identify sustainable management practices, since little is known about the clean-up of MP contamination, coupled with the intricacies of MP extraction, identification, and characterization [41].

2. Materials and Methods

2.1. Study Location

The research was conducted in the greenhouse located within the Department of Plant, Food, and Environmental Sciences, Faculty of Agriculture, at Dalhousie University in Truro, NS, Canada. The study took place from December 2023 to July 2024.

2.2. Microplastics

PE-MPs, PP-MPs, and PS-MPs were obtained from household plastic materials, packaging bags, food packaging containers, and drinking cups with specific identification codes labeled PE, PP, and PS. The plastics were commercially bought from Walmart Supercenter, Truro, NS, Canada, and were cut and fragmented into particle sizes ranging from 1 to 5 mm, equal weights of the sizes were homogenized for the treatments.

2.3. Plant Material and Growing Media

Surface soil (0–20 cm) was obtained from the demonstration garden at the Dalhousie University Agricultural Campus (45°22′15″ N, 63°15′26″ W). The collected soil was screened for rocks and other unwanted materials and stored in the greenhouse until application. Municipal solid waste (MSW)-generated compost was sourced from the Colchester Balefill and Composting facility (Kemptown, NS, Canada), which upcycles organic waste into compost for agricultural and economic purposes.

Tomato cultivar 'Scotia' seeds were obtained from Halifax Seeds (Halifax, NS, Canada). Seeds were sown in a 32-cell pack filled with Pro-Mix® BX (Premier Tech Horticulture, Delson, QC, Canada), and the seedlings were grown for 30 days in a growth chamber. The chamber maintained a day/night temperature of 25 °C, with 16/8 h d^{-1} illumination, 300 µmol $m^{-2} \cdot s^{-1}$ light intensity, and 70% relative humidity. Once the seedlings reached the third to fourth true-leaf stage, uniform seedlings were transplanted into 6.52 L experimental pots, with one seedling per pot. Each experimental pot contained 2 kg of a soil-compost mixture (3:1), with MPs (1% *w*/*w*) at 10 cm depth. The growth medium was climate-hardened for one week before applying the treatments and transplanting, which was performed under greenhouse conditions at a day/night temperature of 28/20 °C and 70% relative humidity with a 16 h photoperiod. Supplemental lighting was provided by a 600 W HS2000 high-pressure sodium lamp with NAH600.579 ballast (P.L. Light Systems, Beamsville, ON, Canada) throughout the growing period.

2.4. Experimental Design and Treatment

The experimental design was a completely randomized design with two (2) treatments, i.e., control (soil and sieved compost without spiked MPs) and M-MPs (soil and sieved compost with 1% *w*/*w* PE-MPs, PS-MPs and PP-MPs) at an equal ratio. The MSW compost was sieved through a 1 mm, 8-inch full-height sieve mesh (Advantech-W.S. Tyler Company,

Moraine, OH, USA) to eliminate potential MPs in compost samples. The sieved compost was added to the soil before the M-MPs were added into the growth media at 10 cm depth. The treatment was applied during the preparation of the growing medium, and regular watering was maintained to field capacity throughout the study. The pots were rearranged biweekly on the bench to mitigate any unforeseen environmental variations within the greenhouse.

2.5. Determination of Plant Growth and Yield

Following the methodology outlined by Ofoe et al. [42], plant growth parameters were evaluated 50 days after transplanting (DAT). Plant height was measured from the stem collar to the highest leaf tip using a measuring ruler, and the main stem diameter or girth was assessed at 10 cm above the stem collar with vernier calipers (Mastercraft®, Toronto, ON, Canada). Intracellular carbon dioxide concentration (C*i*), net photosynthetic rate (A), transpiration rate (E), and stomatal conductance (gs) were measured from four fully expanded leaves per plant, using an LC*i* portable photosynthesis system (ADC BioScientific Ltd., Hoddesdon, UK). Additionally, chlorophyll fluorescence indices, such as maximum quantum efficiency (F_v/F_m) and potential photosynthetic capacity (F_v/F_o), were measured on the same leaves using a chlorophyll fluorometer (Optical Science, Hudson, NH, USA). Chlorophyll content was quantified with a chlorophyll meter (SPAD 502-plus, Spectrum Technologies, Inc., Aurora, IL, USA). The total fresh weight of ripe tomato fruits per plant was measured using a portable balance (Ohaus Navigator®, ITM Instruments Inc., Sainte-Anne-de-Bellevue, QC, Canada). The equatorial and polar diameters of the harvested tomato fruits were measured with a digital Vernier caliper.

2.6. Fruit Analysis

At the time of harvest (75DAT), nine ripe fruits, a representative in size and color, were randomly selected and surface sterilized with 70% ethanol. The pericarp was carefully excised from the longitudinal section of each fruit, using a sterile scalpel blade. The excised pericarp was immediately frozen in liquid nitrogen and stored at −80 °C, while the remaining fruits were preserved at −20 °C for subsequent analyses. The frozen fruits were thawed at room temperature, and the total soluble solids (TSS) were measured with a handheld refractometer (Atago, Tokyo, Japan). The ripe fruits were cut, placed in clear Ziploc bags, and manually squashed to determine TSS. The tomato fruit juice was collected in a 50 mL beaker, with 500 µL used for TSS measurement and expressed as degrees Brix (°Brix). The fruit juice pH, total dissolved solids, and electrical conductivity were measured using a pH/EC/TDS/Temp portable meter (Hanna Instrument, Woonsocket, RI, USA). The elemental composition of the tomato fruits was analyzed at the Nova Scotia Department of Agriculture Laboratory Services in Truro, NS, Canada, using inductively coupled plasma mass spectrometry (PerkinElmer 2100DV, Wellesley, MA, USA) [42,43].

2.7. Biochemical Analysis

2.7.1. Carotenoid Content

Carotenoid content in the fruit was assessed by finely grinding the fruit pericarp following the methodology described by Lichtenthaler [44]. A 0.2 g sample of the ground pericarp was homogenized in 1.5 mL of 80% acetone within a sterile 2 mL Eppendorf tube. The homogenate was then centrifuged at 15,000× g for 15 min. The absorbance of the supernatant was measured at wavelengths of 646.8 nm, 663.2 nm, and 470 nm corresponding to chlorophylls a, b, and carotenoid, respectively, with 80% acetone used as the blank. The total carotenoid content was expressed as µg g^{-1} fresh weight (FW) of the sample.

2.7.2. Total Ascorbate Content

The total ascorbate content was determined following the procedure outlined by Ofoe et al. [42], which is a modified version of the method originally developed by Ma et al. [45]. Approximately 0.2 g of ground fruit pericarp was homogenized in 1.5 mL of ice-cold, freshly prepared 5% trichloroacetic acid (TCA). The resulting mixture was then vortexed for 2 min and subsequently centrifuged at a speed of 12,000× *g* for 10 min at a temperature of 4 °C. A volume of 100 µL of the supernatant was carefully transferred into a new tube and 400 µL of 150 mM phosphate buffer was added. Following this, 100 µL of 10 mM dithiothreitol (DDT) was added to the mixture and vortexed for a duration of 30 s. To initiate the color development, a reaction mixture comprising 400 µL of 10% (*w*/*v*) trichloroacetic acid (TCA), 400 µL of 44% orthophosphoric acid, 400 µL of 4% (*w*/*v*) α, α-dipyridyl in 70% ethanol, and 200 µL of 30 g/L ferric chloride ($FeCl_3$) was added. The resulting mixture was then incubated in a water bath at a temperature of 40 °C for 60 min. Subsequently, the absorbance of the solution was measured at a wavelength of 525 nm. The total ascorbate content was determined by using a standard ascorbic acid curve and was expressed as µmol g^{-1} FW.

2.7.3. Total Phenolics Content

The total phenolic content (TPC) was quantified by adapting the Folin–Ciocalteau assay method [42,46]. Ground fruit pericarp weighing approximately 0.2 g was mixed with 1.5 mL of ice-cold 95% methanol and left to incubate in darkness at room temperature for 48 h. Following this, the mixture was centrifuged at 15,000× *g* for 15 min, after which 100 µL of the supernatant was mixed with 200 µL of 10% (*v*/*v*) Folin–Ciocalteau reagent. The resulting solution was vortexed for 5 min, then mixed with 800 µL of 700 mM sodium carbonate (Na_2CO_3), and allowed to incubate in the dark at room temperature for 2 h. The absorbance of the supernatant was then measured at 765 nm against a blank. TPC was determined using a gallic acid standard curve and reported as mg gallic acid equivalents per gram of fresh weight (mg GAE g^{-1} FW).

2.7.4. Total Flavonoid Content

The quantification of total flavonoid content (TFC) was determined following the colorimetric method outlined by Chang et al. [47]. A 0.2 g sample of ground fruit pericarp was homogenized in 1.5 mL of ice-cold 95% methanol, followed by centrifugation at 15,000× *g* for 15 min. Subsequently, 500 µL of the supernatant was combined with a reaction mixture consisting of 1.5 mL of 95% methanol, 0.1 mL of 10% aluminum chloride ($AlCl_3$), 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The resulting mixture was incubated at room temperature for 30 min, after which the absorbance was measured at 415 nm against a blank devoid of $AlCl_3$. The TFC was determined using the quercetin standard curve and expressed as µg quercetin per gram of fresh weight (µg g^{-1} FW).

2.7.5. Soluble Sugar Content

The determination of the total sugar content of the tomato fruits was carried out following the procedure described by Ofoe et al. [42], which was a modified version of the method originally developed by Dubois et al. [48]. Approximately 0.2 g of ground fruit pericarp was homogenized in 10 mL of 90% ethanol. The resulting mixture was then placed in a water bath at a temperature of 60 °C for 60 min. Subsequently, the final volume of the mixture was adjusted to 5 mL using 90% ethanol and subjected to centrifugation at 12,000× *g* for 3 min. Then, 1 mL aliquot of the supernatant was transferred into a thick-walled glass test tube containing 1 mL of 5% phenol and thoroughly mixed. To initiate the

reaction, 5 mL of concentrated sulfuric acid (H_2SO_4) was added to the mixture, which was then vortexed for 20 s and incubated in the dark for 15 min. The mixture was allowed to cool to room temperature and the absorbance was measured at a wavelength of 490 nm against a blank. Finally, the total sugar content was determined using a standard sugar curve and expressed as μg of glucose g^{-1} FW.

2.7.6. Total Protein Content and Peroxidase Enzyme Activity

The methodology used to assess the fruit protein content and antioxidant enzyme activity was described by Ofoe et al. [42]. A 0.2 g of the ground sample was mixed with 1.5 mL of ice-cold extraction buffer containing 50 mM potassium phosphate buffer (pH 7.0), 1% polyvinylpyrrolidone (PVP), and 0.1 mM ethylenediamine tetraacetic acid (EDTA). The mixture was then homogenized and centrifuged at 15,000× *g* for 20 min at a temperature of 4 °C. The resulting supernatant, representing the crude enzyme extract, was transferred to a new sterile 2 mL microfuge tube on ice. Subsequently, 1 mL of Bradford's reagent was added to the new tube containing the crude enzyme extract, vortexed, and left for 5 min at room temperature before measuring the protein content. A standard curve of bovine serum albumin (BSA), ranging from 100 to 500 μg mL^{-1}, was used to estimate the protein content [49].

For the determination of peroxidase (POD, EC 1.11.1.7) activity, pyrogallol was used as the substrate, following the method described by Maehly [50] and modified by Ofoe et al. [42]. The reaction mixture consisted of 100 mM potassium-phosphate buffer (pH 6.0), 5% pyrogallol, 0.5% H_2O_2, and 100 μL of the crude enzyme extract. After incubation at a temperature of 25 °C for 5 min, the reaction was halted by adding 1 mL of 2.5 N sulfuric acid (H_2SO_4). The absorbance was then measured at a wavelength of 420 nm against double-distilled water (ddH_2O) as the blank. It is noteworthy that one unit of POD forms 1 mg of purpurogallin from pyrogallol in 20 s at pH 6.0 and a temperature of 20 °C.

2.8. Statistical Analysis

The data collected from this study were analyzed using the two-sample *t*-test on Minitab version 21 (Minitab, Inc., State College, PA, USA). Anderson–Darling normality test was first used to ascertain the data normality. Due to the non-normality of some of the data (Anderson–Darling, $p < 0.05$), a Mann–Whitney non-parametric approach was followed [51]. The parameters analyzed were expressed as their median value and their errors were expressed as the interquartile range divided by the square root of the number of observations from five replicates (n = 5).

3. Results

3.1. Physiological Parameters

The results of the chlorophyll content, transpiration rate, photosynthetic rate, sub-stomata CO_2 concentration, and stomatal conductance showed that the M-MPs in the soil had no significant ($p > 0.05$) effect on all physiological attributes measured on the tomato plants. Although the outcomes of both the treatment and the control were comparable, slight differences were noted. Chlorophyll content, transpiration rate, photosynthetic rate and stomata conductance of tomato plants in MP-spiked soil were increased by ca. 5.2%, 16.7%, 25.8% and 20.8%, respectively, compared to the control (Table 1). Conversely, the C*i* value of the tomato plant decreased by ca. 3.23% compared to the control experiment.

3.2. Morphological Response of Tomato to Mixed Microplastics (M-MPs)

There were no significant ($p > 0.05$) effects recorded for the plant height, number of fruits produced, number of leaves, total fruit weight, total root length, root surface area,

root volume, root tips, fruit polar diameter (PD), and equatorial diameter (ED), but there was a significant ($p < 0.05$) effect on the stem girth (Table 2). However, no significant ($p > 0.05$) difference was observed when M-MPs were compared to the control. The plant height, number of leaves, number of fruits, root length, root surface area, and root volume increased non-significantly ($p > 0.05$) by ca. 0.5%, 23.7%, 11.8%, 13.8%, 0.6% and 28.3%, respectively, with M-MPs treatment compared to the control (Table 2). Conversely, M-MPs caused a slight reduction in total fruit weight, fruit PD and ED, and root tips by ca. 0.1%, 4.4%, 7.6% and 13.7%, respectively, compared to the control. In addition, the M-MPs in the soil significantly ($p < 0.05$) caused a 15.0% reduction in the stem girth of the tomato plant.

3.3. Biochemical Activity and Fruit Quality

The biochemical analysis carried out on the harvested tomato fruits revealed significant ($p < 0.05$) effects of M-MPs treatment MPs on carotenoid content, total flavonoid, sugars, total protein, total ascorbate, and peroxidase activity. However, there was no significant ($p > 0.05$) difference between M-MPs treatment and control on the total phenolic content of tomato fruits. Notably, M-MPs caused an increase in the total protein, total phenolics, total ascorbates, and peroxidase activity by ca. 11.1%, 11.5%, 38.4% and 30.2%, respectively, compared to the control (Table 3). Also, the carotenoids, flavonoids and total sugars were reduced in tomato fruits harvested from M-MPs in soil by ca. 12.6%, 42.3% and 21.7%, respectively, compared to the control. Furthermore, the tomato fruit analysis showed a significant ($p < 0.05$) difference in TSS, but no statistically significant difference ($p > 0.05$) was recorded for the TDS and electrical conductivity. Despite this, M-MPs generally caused an increment in all three parameters, TSS (ca. 5.6%), TDS (ca. 9.1%) and EC (ca. 20.0%). In addition, our data on the fruit nutritional values revealed that the presence of the M-MPs [PE-MPs, PS-MPs, and PP-MPs (1:1:1; 1% w/w)] in soil enhanced all the analyzed macronutrients [nitrogen (N), calcium (Ca), potassium (K), magnesium (Mg), phosphorus (P), sodium (Na)] including DM. However, there were some variations in the micronutrient values as M-MPs tomato fruits had higher B and Fe but lower Cu, Mn and Zn values (Table 4).

Table 1. Physiology Parameters of Tomato Plant in m-MP Spiked Soils.

Treatment	SPAD	E (molm^{-2} s^{-1})	A (µmol m^{-2} s^{-1})	C*i* (µmolmol^{-1})	*g*s (molm^{-2} s^{-1})	F_v/F_m	F_v/F_o
Control	60.24 ± 3.90	6.33 ± 2.62	10.81 ± 1.60	342.30 ± 24.40	0.36 ± 0.07	0.79 ± 0.00	3.71 ± 0.45
M-MPs	63.52 ± 2.12	7.60 ± 2.56	14.57 ± 2.30	332.10 ± 46.10	0.45 ± 0.10	0.79 ± 0.00	3.78 ± 0.13
p-value	0.137	0.284	0.196	0.544	0.432	0.383	0.405

SPAD—chlorophyll content; E—transpiration rate; A—photosynthetic rate; *g*s—stomatal conductance; C*i*—Sub-stomatal CO_2. Values are means ± SD of five replicates and a two-sample *t*-test at $\alpha = 0.05$ and 95% confidence level using the Anderson—Darling test for normality. M-MPs = tomato plants in PE+PS+PP-MPs spiked compost with soil.; Control = tomato plants in sieved compost with soil.

Table 2. Morphological Parameters of Tomato Planted in m-MP Spiked Soils.

Treatment	Plant Height (cm)	Number of Leaves	Stem Girth (mm)	Number of Fruits	Total Fruit Weight (g)	Fruit ED (mm)	Fruit PD (mm)	Root Length (cm)	Root Surface Area (cm^2)	Root Tip	Root Volume (cm^3)
Control	55.0 ± 2.33	12.00 ± 1.00	8.71 ± 0.76	12.00 ± 2.55	595.00 ± 133.00	43.42 ± 3.89	37.31 ± 3.02	235.60 ± 45.10	22.12 ± 1.08	7247.00 ± 1145.00	19.5 ± 6.53
M-MPs	55.20 ± 5.71	12.20 ± 1.00	7.41 ± 0.73	13.60 ± 1.95	594.30 ± 93.50	40.11 ± 3.43	35.66 ± 3.40	273.40 ± 30.2	22.26 ±0.57	6253.00 ± 1698.00	27.20 ± 10.6
p-value	0.916	0.651	0.025	0.297	0.997	0.192	0.440	0.158	0.805	0.310	0.202

Fruit ED—Fruit equatorial diameter; Fruit PD—Fruit polar diameter. Values are means ± SD of five replicates and a two-sample *t*-test at $\alpha = 0.05$ and 95% confidence level, using the Anderson–Darling test for normality. M-MPs = tomato plants in PE+PS+PP-MPs spiked compost with soil. Control = tomato plants in sieved compost with soil.

Table 3. Biochemical Activity and Tomato Fruit Juice Quality.

Treatment	Car (μg g^{-1} FW)	TF (μg quercetin^{-1} FW)	TPC (mg GAE g^{-1} FW)	TSC (mg glucose g^{-1} FW)	Total Protein (μg g^{-1} FW)	Total Ascorbate (mM g^{-1} FW)	POD (μg^{-1} FW)	TSS (°Brix)	TDS (g L^{-1})	EC (mS/cm)
Control	0.03 ± 0.00	6.31 ± 0.37	86.63 ± 5.47	2520.00 ± 60.40	5337.00 ± 217.00	53.49 ± 7.99	0.15 ± 0.01	5.07 ± 0.06	1.34 ± 0.01	2.68 ± 0.29
M-MPs	0.03 ± 0.00	3.64 ± 0.26	95.55 ± 1.19	1969.40 ± 37.00	6000.00 ± 168.00	86.80 ± 25.30	0.22 ± 0.01	5.37 ± 0.06	1.48 ± 0.04	3.35 ± 0.48
p-value	0.001	<0.001	0.061	0.030	0.003	0.046	<0.001	0.003	0.642	0.108

TF—total flavonoid; TPC—total phenolic content; TSC—total sugar content; TSS/Brix—total suspended solids; TDS—total dissolved solids; EC—electrical conductivity. Values are means ± SD of five replicates and a two-sample *t*-test at $\alpha = 0.05$ and 95% confidence level using the Anderson–Darling test for normality. M-MPs = tomato plants in PE+PS+PP-MPs spiked compost with soil. Control = tomato plants in sieve compost with soil.

Table 4. Elemental Values of MP-Spiked Tomato.

Treatment	DM (%)	N (ppm)	Ca (ppm)	K (ppm)	Mg (ppm)	P (ppm)	Na (ppm)	B (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)
M-MPs	7.13 *	2.06 *	0.14 *	3.35 *	0.14 *	0.32 *	0.09 *	15.66	6.20	33.71	13.97	12.87
Control	6.67 *	1.92 *	0.11 *	3.15 *	0.13 *	0.31 *	0.09 *	14.84	8.13	28.61	25.15	22.03

DM—dry matter; N—nitrogen; Ca—calcium; K—potassium; P—phosphorus; Na—sodium; B—boron; Cu—copper; Fe—iron; Mn—manganese; Zn—zinc. * = x × 10^3.

4. Discussion

The continuous increase in food demand and the drive for achieving food security and zero hunger has focused on green and environmentally friendly soil management practices such as applying organic composts to agricultural lands to improve soil quality and optimized plant growth, yield, and fruit quality. Hence, farmers and agriculturalists are drawn to the purity and quality of compost as a soil enhancer. In this study, though the contamination of M-MPs had no statistically significant effect on the physiological parameters of the Scotia tomato plant. The treatment caused an insignificant percentage increase in SPAD, transpiration rate, photosynthetic rate, and stomata conductance of the plants. This finding contradicts the results of Wang et al. [52], who reported that MPs reduced the SPAD content of lettuce leaves following foliar application of MPs (Table 1). Specifically, polyethylene microplastics (PE-MPs) have been shown to induce stress and alter enzymatic activity, thereby disrupting chlorophyll synthesis. Consequently, this disruption led to a reduction in chlorophyll content in duckweed (*Lemna minor*). It also exacerbated the toxic effects of cadmium in a co-contamination study [53–55]. Furthermore, PE-MPs have been linked to the reduction in chlorophyll a and b in maize leaves [56]. In a recent study on soybean (*Glycine max*) plants, a concentration of 0.1% PE-MPs resulted in a 6.05% increase in SPAD value [57]. It is possible that the M-MPs treatment did not induce water stress, thereby limiting the production of reactive oxygen species (ROS) that would have disrupted the chlorophyll molecules.

The F_v/F_o ratio shows a plant's potential photosynthetic capacity under optimal conditions, while F_v/F_m shows the maximum quantum efficiency yield, which indicates how efficiently absorbed light is used in photosynthesis, particularly in photosystem II (PSII) [26,58]. In this study, the influence of M-MP on F_v/F_m and F_v/F_o in tomato leaves grown in contaminated soil was negligible (Table 1). Since the F_v/F_m values are expected to be in the range of 0.7–0.8 [59], there was a neutral effect observed which indicated no little or no stress in the plants.

The regulation of stomatal conductance and transpiration rate is essential for plants to efficiently control gas exchange and water use in response to environmental conditions, which in turn affects their ability to regulate temperature and perform photosynthesis [42,60]. This study demonstrated that M-MPs slightly improved gs and E. This increase in E aligns with a rice-MPs interaction study using 3 mg/L PS-MPs but contradicts with gs and SPAD which experienced a 33.6% and 34.9% decline, respectively [61]. Although there is limited direct research on the effects of M-MPs on the physiological traits of tomato plants, it can be hypothesized that the presence of microplastics may enhance photosynthesis and nutrient uptake in tomato plants and provide some protection against stress. This is because plants often reduce stomatal conductivity as an adaptive strategy to manage water loss, heat stress, and other related climatic stressors [42]. Conversely, increased stomatal conductance and transpiration can also lead to greater water loss.

Similarly, the photosynthetic rate (A) showed a significant increase with M-MPs treatment. This finding contradicts the results of Ma et al. [61], who reported that PS-MPs reduced the photosynthetic rate by 31.5%. The reduction was less pronounced at a lower concentration of PS-MPs (0.5 mg/L), with a decrease of only 11.8%. The results also contradicted Li et al. [26], who observed a decline in photosynthetic pigments in cucumber (*Cucumis sativus*) leaves exposed to PS nanoparticles (100 nm and 700 nm). These discrepancies might be attributed to the nano size and concentration of the plastic particles, which could facilitate the absorption of PS-NPs into plant leaves. Additionally, the presence of PE-MPs and PP-MPs may have mitigated the negative effects of MPs on tomato plants. This suggests that the 1% w/w concentration used in this study might have contributed to the observed enhancement in the photosynthetic rate of tomato plants. In the same study

on rice, the internal CO_2 concentration (C*i*) decreased with 3 mg/L PS-MPs, a finding that is consistent with the slight reduction in C*i* observed with M-MPs treatments in this study.

Agricultural scientists and farmers are interested in plant productivity, i.e., fruit yield, and the present study demonstrated that M-MPs have no significant effect on tomato fruit yield and size (including fruit weight, fruit ED and PD). Given that the physiological activities of the plants were unaffected by M-MPs, it could be hypothesized that the tomato yield from the treatment is comparable with the control experiment. As seen in Table 2, comparable fruit weights were recorded, M-MPs slightly increased the number of fruits but caused a decline in the fruit size including fruit weight, fruit ED, and PD. This result aligns with a recent study that reported a reduction in the number of tomato fruits produced by PET-MPs and PVC-MPs pollutants [62]. Outstandingly, M-MPs caused a significant decline in the tomato stem girth. The stem girth is the circumferential measurement of the stem that could be linked to the thickness of the plant, stamina and vigor. The importance of the stem girth in tomato is mostly evident at the fruiting stage where the plant requires more support for the weight of the fruits. Thinner stems could bend, break, and cause damage to plant vascular tissues. Also, stem girth is principal in the translocation of water and nutrients [63]. This conforms with a recent study on the ability of PP-MPs to reduce the translocation of macronutrients in tomato plants [64]. M-MPs treatment had negligible effects on the tomato plant height confirming [62] that PET-MPs and PVC-MP had insignificant effects on plant growth.

Similarly, the root morphology demonstrated negligible differences though the M-MPs spiked plants slightly improved the root length, surface area and volume (Table 2). Contradictorily, it caused a reduction in the number of root tips produced. The inversely proportional relationship between the root volume and root tips is suggested to be a devised means for the plant to make up for adequate nutrient uptake from the soil. Zhuang et al. [65] reported that MPs have the potential to significantly obstruct root growth in cucumber plants, but this study demonstrated that the effects of MPs in soil can be inconsequential to root growth in tomato plants. Contrary to the effect of PE-MPs on strawberry (*Fragaria ananassa*) roots [66], PS-MPs (0.3 mm) have been reported to significantly increase the root profile of cucumber plants [67].

This study was monitored until fruit harvesting, and this allowed for the evaluation of some antioxidant compounds in tomato fruits to understand how plastic contaminants in soil could affect fruit quality. The carotenoid and flavonoid content measured in tomato fruits harvested from growth media containing the combination of MPs was drastically lower than those in the control group, with a statistically significant effect. On the contrary, M-MPs significantly increased the ascorbate content, total protein content, POD activity and TSS. These POD activity changes contradict the effect of PVC and PS on cucumber. PVC and PS-MPs were reported to decrease the POD activity in cucumber plants [65]. However, in the presence of a combination of PE-MPs, PS-MPs, and PP-MPs, there was a percentage increase in TDS and EC. These parameters are important in determining the quality of fruits, this suggests that the combination of PE-MPs, PS-MPs and PP-MPs in soil could have a positive contribution to improving food security and safety. However, due to the decline in carotenoids and flavonoids caused by M-MPs treatment, it is difficult to place the combinative effect of PE-MPs, PS-MPs and PP-MPs on fruit quality on a positive scale since carotenoids and flavonoids are part of the antioxidants and health benefit strength of tomato [42]. This result conforms with [62] which reported a decline in lycopene and phenolics. This reduction may be associated with MP types that have a greater propensity to generate oxidative stress in plants. The coloration of fruit is dependent on its carotenoid concentration, which is associated with pigmentation in fruits [68], and prevention against oxidative stress [42], hence critical to customer preference, and human and plant health.

Contrary to [62] report on PET-MPs, M-MPs-treated plants produced fruits with increased TSS. TSS is regarded as a key factor considered by consumers [69].

Another significant effect was recorded on the POD of tomato fruits from M-MPs treated plants. Peroxidase activity is closely related to the fruit's quality and resistance to stress and pathogens since it is associated with physiological and biochemical processes in plants [70]. POD also possesses reactive oxygen species (ROS)-scavenging abilities that protect the cells against oxidative stress [42]. Other notable increments observed in M-MPs fruits include TDS and EC. While EC can be associated with fruit quality (flavor and firmness) and sugar content [71], it could also be linked to worsening biochemical compounds [72], calcium deficiency, causing blossom end rot on fruits [71]. This was experienced during this study, although no data were collected on this deficiency. EC has also been connected to the interference of some essential nutrient uptake [72]. These phytochemicals have been reported to be connected to the prevention of cancers, atherosclerosis and inflammatory diseases [24,42,73,74]. Reduction in the quantities of carotenoids and flavonoids contained in tomato fruits could jeopardize the fruit's ability to reduce the risk of the mentioned health issues.

While the number of fruits made up for the smaller size, both the farmers' and consumers' satisfaction are only partially included in these results. This study anticipated no increase in antioxidants in the microplastics-spiked fruits, since there were negligible effects on the plant's physiological traits and overall growth. This inconsistency could be linked to the expression of stress since the plants store up these phytochemical compounds as an adaptation strategy to stress. However, there remains a lack of scientific evidence to support how MP-induced plant stress could influence plant metabolic activities and overall performance. Additionally, the variability in the reaction of morpho-physiological traits and phytochemical traits could also be due to the sample size and random selection of the fruits used in the analysis. An increase in TDS, sugar, and proteins in tomato fruits has been reported to be associated with tomato plant response to salinity stress [75,76]. Consequently, it is plausible that although M-MPs-spiked soil did not significantly alter the overall physiology and morphology of the tomato plants, it instigated the plants to store up these biochemicals in the fruits.

The nutritional composition of fruits is a key factor in determining the quality of fruits, and the elemental content of the M-MPs-spiked tomato fruits aligns with the phytochemical contents which make up a small percentage of a fruit's dry matter, yet they are essential for enhancing the quality and nutritional value of vegetables. Despite their minor presence in the overall composition, these minerals play a crucial role in contributing to the health benefits and flavor profiles that vegetables offer [42,77]. Some potential reasons might include (1) soil pH status and deficiency in Cu, Mn, and Zn; (2) antagonistic interactions as a result of high levels of P availability; (3) contaminations of heavy metals such as lead (Pb) or cadmium (Cd) in the soil could inhibit the uptake of essential metals; and (4) organic compounds in polymers, which may interfere with the availability of these micronutrients (undetermined). In a recent study, 0.2% of PP-MPs caused an increase in Fe levels while PVC-MPs decreased the Fe content in roots. PE-MPs also increased the Ca levels [78]. PP-MPs and PE-MPs also increased Cu, Mn, and Zn levels in the field pumpkin (*Cucurbita pepo*) roots contrary to this study where the M-MPs decreased Cu, Mn, and Zn in tomato fruits [78]. Although it was anticipated that the presence of MPs would increase the Cu availability as reported in Pinto-Poblete et al. [66], the combination of PS-MPs and PP-MPs to the treatment could be a plausible explanation for the contradictory effect that was observed.

5. Conclusions

The effect of combined PE, PS, and PP MPs on the growth, yield, and biochemical properties of tomato plants were evaluated in the present study. These MP types are prevalent in compost which is commonly used as a soil enhancer. Although the mixture of these MPs did not significantly affect the physio-morphological traits and yield parameters of the tomato 'Scotia' plants, some outstanding changes were observed in the biochemical activities and tomato fruit quality. Specifically, the M-MPs treatment led to an increase in the total ascorbate, total protein content, peroxidase activity, and total soluble solids, indicating an adaptive stress response in the tomato plants. However, a noticeable reduction was observed in the carotenoid and flavonoid content of the tomato fruits. This raised concerns about the potential impact of microplastics on the nutritional quality and antioxidant capacity of fruits. In summary, these findings suggest that while microplastics in soil may not drastically harm the growth or yield of tomatoes, their presence could alter fruit quality by reducing the key antioxidants. It is recommended that future studies focus on the soil with long-term exposure to microplastics, varying concentrations of the different microplastic types, smaller MP sizes (<1 mm), and the response of different plant species. Also, future analysis should conduct the experiment at least three times to eliminate bias from sample sizes and distribution. Furthermore, as MPs continue to accumulate in the soil, their potential effects on plant health and food quality must be prioritized to mitigate the possible risks associated with food and nutrition security. It is important to conduct a long-term exposure study of MPs in soil on tomatoes, considering possible desorption and uptake of some polymer derivatives.

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