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A Comparative Study on the Effects of Nano- and Microplastics on Nutrient Element Accumulation and Physiological Performance in Arugula (Eruca sativa L.): Implications for Food Crop Safety

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ABSTRACT

Plastic pollution in irrigation water poses an emerging threat to agricultural systems. This study compared the effects of nanoplastics (NPs, 248 nm) and microplastics (MPs, 34 µm) on arugula (Eruca sativa L.) exposed to 10–100 mg/L concentrations justified by Tigris River contamination modeling. Plants were cultivated for 65 days in controlled chambers; particle characterization employed Py-GC/MS, µ-Raman, TEM-EDS, and confocal microscopy. Nitrogen was quantified by Kjeldahl digestion, other minerals by ICP-OES. Statistical analysis used linear mixed-effects models with Benjamini–Hochberg correction. Nanoplastics induced greater nutrient depletion than microplastics: at 100 mg/L, nitrogen declined 50.9% (NP) vs. 37.3% (MP), iron 55.4% (NP) vs. 34.8% (MP) ($P < 0.001$). Oxidative stress markers (H_2O_2, MDA) increased 189% and 201% under NP vs. 156% and 168% under MP ($P < 0.001$). Although MP root accumulation (42.4 mg/kg) exceeded NP accumulation (14.4 mg/kg) threefold, NPs proved more phytotoxic. Subcellular imaging revealed NP penetration into chloroplasts and mitochondria; MPs remained extracellular. Fresh biomass declined 58.2% (NP) vs. 41.7% (MP) ($P < 0.001$). Antioxidant enzyme upregulation was stronger under NP exposure but insufficient to prevent oxidative damage. These findings demonstrate that nanoplastics pose a greater physiological threat to arugula than microplastics through organellar penetration and cellular dysfunction, despite lower tissue accumulation. Results highlight the urgent need to incorporate nano-scale particles into irrigation water quality standards and treatment technologies.

1-Introduction

The global proliferation of plastic pollution has emerged as one of the most pressing environmental challenges of the twenty-first century. Global plastic production reached approximately 390.7 million tons in 2021, with an estimated 60% of cumulative plastic waste persisting in the environment [1]. Upon environmental exposure, plastic debris undergoes fragmentation through ultraviolet radiation, mechanical abrasion, chemical oxidation, and biological processes, yielding microplastics (MPs; 0.1 µm – 5 mm) and nanoplastics (NPs; < 0.1 µm or < 1 µm depending on classification frameworks) [2, 3]. While MPs have received considerable research attention, NPs exhibit fundamentally distinct environmental behaviors due to their nanoscale dimensions, substantially higher surface-area-to-volume ratio, enhanced surface reactivity, and superior mobility across porous media and biological barriers [3, 4]. Agricultural ecosystems represent a major sink for plastic contamination. Primary sources include plastic mulching films, sewage sludge application, composted fertilizers, irrigation water, and atmospheric deposition [2, 5]. Contaminated irrigation water has been identified as a critical yet understudied pathway for introducing plastic particles into croplands [6, 7]. Upon entry into agroecosystems, MNPs (micro- and nanoplastics) can alter soil physicochemical properties, disrupt microbial community structure, and directly interact with plant root systems [1, 2]. Vegetable crops are particularly vulnerable due to their high transpiration rates, short growth cycles, and consumption as fresh produce [8].

Plants internalize MNPs primarily through root uptake via apoplastic and symplastic pathways, crack-entry at lateral root junctions, and, for NPs, endocytosis [2, 9]. Following internalization, NPs can translocate systemically through vascular tissues and accumulate in edible above-ground organs, including leaves and fruits [3, 4]. This pathway raises profound concerns for food safety and human health, particularly given recent reports documenting plastic particle accumulation in human tissues including kidney, liver, and brain [4].

The phytotoxicity of MNPs is predominantly mediated by reactive oxygen species (ROS)-induced oxidative stress [1, 10]. Excess ROS generation provokes lipid peroxidation, protein oxidation, DNA damage, and ultimately cellular dysfunction [11]. Concomitantly, MNP exposure impairs photosynthetic efficiency, disrupts mineral nutrient homeostasis, and suppresses biomass accumulation [2, 12]. Critically, the magnitude of these effects appears size-dependent: NPs demonstrate enhanced capacity to penetrate cellular and subcellular compartments—including chloroplasts and mitochondria—relative to their micro-scale counterparts [4, 13].

Among vegetable crops, members of the Brassicaceae family are of particular significance due to their global dietary importance and documented sensitivity to environmental contaminants [14]. Arugula (Eruca sativa L.) is a rapidly growing leafy vegetable widely consumed in Mediterranean diets and increasingly cultivated worldwide [15]. Its nutritional profile—rich in glucosinolates, flavonoids, and essential minerals—combined with its short production cycle renders it an ideal model species for assessing contaminant impacts on crop quality and food safety. Previous investigations have established that E. sativa exhibits pronounced physiological responses to abiotic stressors including heavy metals, characterized by photosynthetic inhibition, oxidative damage, and antioxidant enzyme modulation [15, 16]. However, despite the escalating threat of plastic pollution in irrigation water, comparative data on the differential effects of nano- and microplastics on mineral nutrition and physiological performance in this species remain absent.

Accordingly, the present study was designed to provide a systematic comparative assessment of nanoplastics (248 nm) versus microplastics (34 µm) on arugula cultivated under controlled environmental conditions. Exposure concentrations (10, 50, and 100 mg/L) were selected based on contamination scenario modeling of the Tigris River basin, representing environmentally relevant irrigation water contamination levels. We employed an

integrated analytical approach encompassing particle characterization (Py-GC/MS, μ-Raman spectroscopy, TEM-EDS, confocal microscopy), comprehensive mineral nutrient quantification (Kjeldahl nitrogen and ICP-OES), and physiological stress marker analysis. We hypothesized that NPs would induce disproportionately greater phytotoxicity than MPs despite lower tissue accumulation, attributable to their capacity for subcellular penetration and consequent disruption of organellar function. By elucidating size-dependent mechanisms of MNP phytotoxicity in a commercially relevant leafy vegetable, this study aims to inform evidence-based revisions to irrigation water quality standards and contribute to the development of targeted mitigation strategies for safeguarding food crop safety in an era of pervasive plastic pollution.

2- MATERIALS AND METHODS

2.1 Experimental Site and Design

2. Materials and Methods

2.1 Experimental Design and Growth Conditions

The experiment was conducted at Samarra University, College of Education, Department of Biology, Iraq (34.1890°N, 43.8703°E), utilizing four Conviron PGW40 controlled-environment growth chambers. All chambers were maintained under identical conditions: 24 ± 1°C day/18 ± 1°C night, 65 ± 5% relative humidity, 14-h photoperiod, and photosynthetically active radiation of 480 ± 30 μmol m^{-2} s^{-1}.

A two-way factorial design was employed with growth chamber as a random blocking factor. Fixed factors comprised plastic type (control, nanoplastic, microplastic) and concentration (10, 50, 100 mg/L). Five replicate plants per treatment were allocated to each of the four chambers. To minimize positional bias, treatment assignments were randomized among chambers with systematic plant rotation every 7 days and complete chamber reassignment every 14 days. Pre-experimental chamber uniformity verification confirmed coefficients of variation <5% for light intensity, temperature, and humidity across nine positions per chamber (one-way ANOVA, $P > 0.05$).

2.2 Plastic Particle Preparation and Characterization

2.2.1 Particle Generation

Polyethylene terephthalate (PET) bottles (commercial mineral water, Samarra source) were fragmented into 1–2 cm pieces, rinsed with distilled water, and subjected to mechanical ball milling (FRITSCH Pulverisette 7, Germany) for 8 h in absolute ethanol using ceramic media. Microplastic (MP) fractions (1–100 μm) were isolated via sieve shaker (Retsch AS 200, Germany).

Nanoplastic (NP) particles were generated through accelerated UV-driven oxidation of MP fragments. Microplastic suspensions (10 g/L in distilled water) were exposed to UV-C radiation (λ = 254 nm, Vilber-Lourmat mercury vapor lamp, France) for 4 h daily at 20 cm distance over 14 consecutive days. Following UV treatment, suspensions underwent cascade centrifugation: primary centrifugation at 1,000 × g for 10 min removed particles >10 μm; secondary centrifugation at 10,000 × g for 30 min eliminated residual MP fractions (100–1000 nm). The NP-enriched supernatant was concentrated via ultrafiltration (Amicon Ultra-4, 100 kDa MWCO, Millipore, USA).

2.2.2 Physicochemical Characterization

Particle size distribution and surface charge were determined in local Samarra groundwater matrix (salinity 0.8 dS/m, pH 7.4, total hardness 285 mg/L $CaCO_3$) using dynamic light scattering (DLS; Malvern Zetasizer Nano ZS, UK). Measurements were performed at three time points: T_0 (immediate post-preparation), T_{24} (24 h static), and T_{72} (72 h with intermittent sonication). At T_0, Z-average diameters were 34.2 ± 3.8 μm (PDI 0.31 ± 0.04) for MPs and 248 ± 42 nm (PDI 0.28 ± 0.05) for NPs. Zeta potential values were −18.4 ± 2.1 mV and −42.1 ± 3.8 mV for MP and NP, respectively.

Transmission electron microscopy (TEM; JEOL JEM-2100, Japan) at 200 kV confirmed MP modal size range of 25–45 μm and NP modal range of 180–320 nm, with no detectable cross-contamination between fractions.

Fourier-transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR; Perkin Elmer Spectrum Two, Germany) verified PET polymer identity through characteristic absorption bands: 1712 cm^{-1} (C=O stretching), 1241 and 1093 cm^{-1} (C–O stretching), and 881 cm^{-1} (aromatic C–H out-of-plane deformation).

2.3 Plant Material, Growth Substrate, and Treatment Application

Arugula seeds (Eruca sativa L., cv. 'Mediterranean Rocket', Al-Rasheed Seeds Company, Baghdad, Iraq) were surface-sterilized with 0.5% NaOCl for 2 min, thoroughly rinsed, and germinated in a 1:1 (v/v) peat:perlite mixture. Three-day-old seedlings were transplanted into 2-L vessels containing modified peat-perlite substrate amended with basal nutrients.

Control plants received unamended local groundwater (salinity adjusted to 0.8 dS/m). Treated plants received identical groundwater supplemented with PET microplastic or nanoplastic particles at final concentrations of 10, 50, or 100 mg/L. All treatments received weekly application of modified Hoagland nutrient solution (1 L per plant) containing: N 6.0, P 1.0, K 6.0, Ca 4.0, Mg 2.0, S 2.0 mM, and Fe 0.09, Zn 0.004, Cu 0.002, B 0.05, Mn 0.009, Mo 0.0005 mM. Irrigation (1.5 L every 3 days) commenced at the second true leaf stage (8 days post-sowing) and continued throughout the 65-day experimental period.

2.4 Experimental Duration and Harvest

Plants were cultivated for 65 days post-germination, corresponding to the late vegetative/pre-flowering stage (10–12 true leaves; incipient bolting evident in controls) and representing the commercial harvest window for arugula. Final harvest was conducted at day 65 for all analyses.

2.5 Analytical Determinations

2.5.1 Mineral Element Analysis

Fully expanded leaves (third leaf from apical meristem) were harvested, rinsed, blotted dry, and lyophilized (Labconco FreeZone 4.5, USA) for 48 h. Total nitrogen was determined by Kjeldahl digestion according to ISO 6737:2018. Dried tissue (0.2 g) was digested with 15 mL concentrated H_2SO_4 and 0.5 g selenium catalyst at 420°C for 2 h. Following distillation (Gerhardt Vapodest 30S, Germany), ammonia was captured in boric acid and titrated with 0.1 M HCl. Nitrogen content (%) was calculated as: (V_sample − V_blank) × N_HCl × 0.014 / sample weight × 100.

For other elements, lyophilized samples (0.5 g) were digested in 70% nitric acid (Suprapur grade) using microwave digestion (CEM MARS Xpress, USA) at 180°C, 1200 W for 30 min. Digests were diluted to 50 mL with deionized water and analyzed for P, K, Ca, Mg, Fe, Zn, Cu, Mn, B, and Mo by inductively coupled plasma optical emission spectroscopy (ICP-OES; Perkin Elmer Optima 8300, USA). Calibration utilized NIST-traceable multi-element standards. Analytical accuracy was verified using certified reference material (tomato leaves, NIST SRM 1570a); duplicate analysis of 20% of samples yielded recoveries within ±5% of certified values.

2.5.2 Photosynthetic Gas Exchange and Chlorophyll Fluorescence

Net photosynthetic rate (P_n), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and transpiration rate (E) were measured on the third fully expanded leaf using a portable photosynthesis system (LiCOR LI-6800F, USA) under standardized conditions: 400 ppm CO_2, 1000 μmol m^{-2} s^{-1} photosynthetically active radiation, 25°C leaf temperature, and 60% relative humidity. Measurements were conducted between 09:00–11:00 h to minimize diurnal variation.

Maximum quantum efficiency of photosystem II (Fv/Fm) was determined using a pulse-amplitude modulation fluorometer (WALZ PAM-2500, Germany) following 30 min dark adaptation. Effective quantum yield (ΔF/Fm') was measured under actinic illumination.

2.5.3 Chlorophyll and Pigment Quantification

Relative chlorophyll content was estimated non-destructively using a portable chlorophyll meter (Konica Minolta SPAD-502 Plus, Japan), with five measurements per leaf. For pigment extraction, fresh leaf tissue (0.5 g) was homogenized in 10 mL 80% acetone,

centrifuged, and absorbance measured at 470, 645, and 663 nm (Shimadzu UV-1700 spectrophotometer, Japan). Pigment concentrations were calculated using Lichtenthaler extinction coefficients [17].

2.5.4 Oxidative Stress Biomarkers and Antioxidant Enzyme Assays

Fresh leaf tissue (0.5 g) was ground in liquid nitrogen and extracted with 0.1 M potassium phosphate buffer (pH 7.4). Homogenates were centrifuged at 12,000 × g for 15 min at 4°C.

Hydrogen peroxide (H_2O_2) content was determined by the titanium(IV) oxysulfate method [18]. Absorbance was measured at 415 nm ($\varepsilon = 0.28$ μmol^{-1} cm^{-1}); results expressed as μmol g^{-1} fresh weight (FW). Malondialdehyde (MDA) concentration was quantified via thiobarbituric acid reaction [19], with absorbance corrected at 532 and 600 nm (ε = 155 mM^{-1} cm^{-1}); results expressed as μmol g^{-1} FW.

Antioxidant enzyme activities were assayed spectrophotometrically and expressed as units (U) mg^{-1} protein. Protein concentration was determined by Bradford assay using bovine serum albumin as standard.

Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured by inhibition of nitroblue tetrazolium (NBT) photochemical reduction at 560 nm [20]. One unit was defined as the enzyme quantity causing 50% NBT inhibition per minute. Catalase (CAT; EC 1.11.1.6) activity was assayed by monitoring H_2O_2 decomposition at 240 nm [21]; $\varepsilon = 39.4$ mM^{-1} cm^{-1}; one unit = 1 μmol H_2O_2 degraded min^{-1}. Peroxidase (POD; EC 1.11.1.7) activity was determined via guaiacol oxidation at 470 nm; $\varepsilon = 26.6$ mM^{-1} cm^{-1}; one unit = 1 μmol guaiacol oxidized min^{-1}. Ascorbate peroxidase (APX; EC 1.11.1.11) activity was measured by ascorbate oxidation at 290 nm; $\varepsilon = 2.8$ mM^{-1} cm^{-1}; one unit = 1 μmol ascorbate oxidized min^{-1}.

2.5.5 Quantification of Plastic Accumulation in Plant Tissues

2.5.5.1 Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS) – Primary quantification

Lyophilized tissue (50 mg) was pyrolyzed at 700°C (Frontier Laboratories EGA/PY-3030D coupled to Shimadzu GC-2010 Plus, Japan). PET-specific pyrolysis products—ethylene glycol monoacetate (m/z 104), benzoic acid (m/z 122), and dimethyl terephthalate (m/z 194)—were quantified against linear calibration curves constructed from certified PET standards (5–500 µg; Sigma-Aldrich). Results expressed as mg PET kg^{-1} dry weight (DW). Recovery rates of PET spikes (10–100 µg) in tissue matrix ranged from 94% to 98%.

2.5.5.2 Nile Red Fluorescence – Secondary confirmation

Fresh tissue (100 mg FW) was incubated in 10 µg mL^{-1} Nile Red solution (ethanol) for 30 min in darkness at 25°C. Following ethanol rinse and air-drying, fluorescence was measured at λ_ex = 480 nm, λ_em = 550–600 nm (Turner Designs Aquafluor, USA). Plastic burden was calculated from PET calibration standards (5–200 µg mL^{-1} ethanol).

2.5.5.3 Micro-Raman Spectroscopy – Spatial distribution

Ultramicrotome sections (10 µm) of resin-embedded tissues were examined by confocal Raman microscopy (Renishaw inVia, UK; 532 nm laser, 2 µm lateral resolution). PET-specific Raman bands at 1611 cm^{-1} (aromatic C=C), 1505 cm^{-1} (aromatic C=C out-of-plane), and 1408 cm^{-1} (C–H bending) were mapped. Intensity ratios at 1611 cm^{-1} correlated linearly with Py-GC/MS quantitation ($R^2 = 0.87$).

2.5.6 Subcellular Localization of Plastic Particles

2.5.6.1 Confocal Laser Scanning Microscopy (CLSM) with Fluorescent Labeling

Nile Red-labeled NP and MP preparations were administered to a parallel plant cohort (n = 6). Fresh leaf tissues were examined immediately post-harvest using CLSM (Zeiss LSM 880 with Airyscan, Germany) with excitation at 488 nm (2% transmission) and emission collected at 500–550 nm. Z-stack optical sections (2 µm per slice, 30 µm depth) were acquired. Nile Red fluorescence (red punctate signals) was co-localized with chlorophyll autofluorescence

(λ_ex = 405 nm, λ_em = 650 nm) to confirm organellar penetration.

2.6.6.2 Correlative Transmission Electron Microscopy–Energy Dispersive Spectroscopy (TEM-EDS)

Ultrathin sections (70–90 nm) were examined by TEM (JEOL JEM-2100, Japan) coupled with an EDS detector (Oxford Instruments, UK). Elemental composition of electron-dense intracellular particles was analyzed. Atomic C/(C+O+N) ratios were calculated and compared with PET reference values (0.59 ± 0.04).

2.5.6.3 Micro-Raman Spectroscopy on Ultra-thin Sections

Ultramicrotome sections (10 μm) were subjected to micro-Raman mapping (Renishaw inVia, 532 nm, 2 μm resolution). PET-specific Raman signatures were spatially distinguished from background tissue signals (cellulose: ~1095, 1375 cm^{-1}; protein amide I: ~1660 cm^{-1}).

2.5.7 Growth and Biomass Parameters

At final harvest (day 65), plants were separated into aerial and root portions. Fresh weights were recorded immediately; dry weights were determined after oven-drying at 65°C to constant mass. Plant height was measured from soil surface to apical meristem. Leaf area was quantified using a leaf area meter (LI-COR LI-3100C, USA). Specific leaf area (SLA) was calculated as leaf area per unit leaf dry weight. Relative growth rate (RGR) was calculated as $RGR = (\ln W_2 - \ln W_1)/(t_2 - t_1)$, where W represents total dry weight at day 45 and day 65, and t represents time interval.

2.6 Statistical Analysis

All statistical analyses were performed using R version 4.3.2 [22] with packages lme4, emmeans, and multcomp.

Normality of scaled residuals was assessed using Shapiro–Wilk test; homogeneity of variance was evaluated via Levene's test. Where assumptions were violated, data were transformed using log or Box–Cox transformations, with transformation methods documented per variable.

Linear mixed-effects models were constructed as:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + \varepsilon_{ijk}$$

where α_i represents plastic type (control, NP, MP), β_j represents concentration (10, 50, 100 mg/L), (αβ)_{ij} denotes interaction term, γ_k represents random effect of growth chamber (k = 1–4), and ε_ijk is residual error. Five replicate plants per treatment were nested within each chamber.

Multiple comparisons were conducted using Tukey's Honest Significant Difference (HSD) test. Benjamini–Hochberg false discovery rate (FDR) correction was applied across all physiological endpoints (α = 0.05). Effect sizes were reported as Hedges' g (bias-corrected Cohen's d) with 95% confidence intervals and partial eta-squared (η^2_partial) for fixed effects.

Dose–response relationships were modeled using non-linear mixed-effects models (nlme package) fitted with a three-parameter sigmoidal function:

$$Y = \frac{Y_{max}}{1+\exp(-(Conc-x_0)/b)} + \varepsilon$$

Model parameters were compared between plastic types using likelihood ratio tests. Model fit was evaluated via root mean square error (RMSE) and Akaike Information Criterion (AIC).

3- RESULTS

3.1 Plastic Particle Characterization

Transmission electron microscopy (TEM) confirmed distinct size distributions for nanoplastic (NP) and microplastic (MP) preparations. Microplastic particles exhibited a size range of 1.2–98.5 μm, with a mean diameter of 32.1 ± 18.4 μm (n = 200 particles). Nanoplastic particles ranged from 12 to 987 nm, with a mean diameter of 287 ± 156 nm (n

= 500 particles; 90th percentile confidence interval: 156–398 nm). Dynamic light scattering (DLS) in aqueous suspension corroborated these findings, yielding modal diameters of 45.3 ± 12.1 μm for MPs and 318 ± 89 nm for NPs. Fourier-transform infrared spectroscopy (FTIR) confirmed PET polymer composition for both preparations, with characteristic absorption bands at 1712, 1241, 1093, and 881 cm^{-1}. Cascade centrifugation coupled with ultrafiltration achieved effective size fractionation, with no detectable cross-contamination between NP and MP fractions.

3.2 Leaf Mineral Element Concentrations

Exposure to microplastics and nanoplastics induced divergent patterns of mineral nutrient depletion, with nanoplastics exerting significantly greater adverse effects across all elements analyzed (Table 1).

Total nitrogen content, determined by Kjeldahl digestion, was 3.42 ± 0.12% dry weight (DW) in control plants. Following exposure to 100 mg/L nanoplastics, nitrogen content declined to 1.68 ± 0.09% DW, representing a 50.9% reduction ($F_{2,30}$ = 142.6, P < 0.001). Microplastic exposure at the same concentration reduced nitrogen to 2.14 ± 0.09% DW (37.3% decrease). The difference between NP and MP treatments was statistically significant ($F_{1,30}$ = 78.4, P < 0.001). Phosphorus concentration in control plants was 1.12 ± 0.08% DW. Nanoplastic treatment at 100 mg/L reduced phosphorus to 0.52 ± 0.04% DW (53.6% decrease), whereas microplastic treatment yielded 0.71 ± 0.05% DW (36.6% decrease; $F_{2,30}$ = 124.8, P < 0.001). Potassium followed a comparable pattern: control values of 3.24 ± 0.16% DW declined to 1.48 ± 0.10% DW under NP exposure (54.3% decrease) and to 2.11 ± 0.12% DW under MP exposure (34.9% decrease; $F_{2,30}$ = 142.6, P < 0.001). Micronutrient analysis revealed pronounced iron depletion. Control plants contained 92 ± 5 mg kg^{-1} DW iron, which decreased to 41 ± 3 mg kg^{-1} under NP treatment (55.4% decrease) and to 60 ± 4 mg kg^{-1} under MP treatment (34.8% decrease; $F_{2,30}$ = 118.2, P < 0.001). Zinc exhibited the most substantial proportional reduction: control values of 54 ± 3 mg kg^{-1} DW declined to 21 ± 2 mg kg^{-1} under NP exposure (61.1% decrease) and to 34 ± 2 mg kg^{-1} under MP exposure (37.0% decrease; $F_{2,30}$ = 135.7, P < 0.001).

Linear regression analysis demonstrated strong negative correlations between nanoplastic concentration and all mineral elements (R^2 = 0.92–0.96), with regression slopes significantly steeper than those observed for microplastic treatments (R^2 = 0.84–0.89).

3.3 Photosynthetic Performance and Chlorophyll Content

Net photosynthetic rate (P_n) in control plants averaged 18.3 ± 0.7 μmol CO_2 m^{-2} s^{-1}. Exposure to 100 mg/L nanoplastics reduced P_n to 7.4 ± 0.5 μmol CO_2 m^{-2} s^{-1}, a 59.6% decline ($F_{2,30}$ = 168.4, P < 0.001). Microplastic treatment at the same concentration yielded P_n of 10.6 ± 0.6 μmol CO_2 m^{-2} s^{-1} (42.1% decrease; $F_{1,30}$ = 127.3, P < 0.001). Stomatal conductance (g_s) decreased from control values of 0.38 ± 0.02 mol m^{-2} s^{-1} to 0.12 ± 0.01 mol m^{-2} s^{-1} under NP exposure (68.4% decrease; $F_{2,30}$ = 174.9, P < 0.001) and to 0.18 ± 0.01 mol m^{-2} s^{-1} under MP exposure (52.6% decrease; $F_{1,30}$ = 156.2, P < 0.001).

Relative chlorophyll content, estimated via SPAD, declined from 44.2 ± 1.6 units in controls to 18.4 ± 1.2 units under NP treatment (58.4% decrease; $F_{2,30}$ = 156.8, P < 0.001) and to 26.8 ± 1.3 units under MP treatment (39.4% decrease; $F_{1,30}$ = 134.6, P < 0.001). Maximum quantum efficiency of photosystem II (Fv/Fm) was significantly impaired by plastic exposure. Control plants exhibited Fv/Fm of 0.794 ± 0.018, which decreased to 0.512 ± 0.020 under NP treatment (35.5% decrease; $F_{2,30}$ = 89.3, P < 0.001) and to 0.631 ± 0.019 under MP treatment (20.5% decrease; $F_{1,30}$ = 92.4, P < 0.001).

3.4 Oxidative Stress Indicators

Hydrogen peroxide (H_2O_2) content in control leaves was 0.72 ± 0.04 μmol g^{-1} fresh weight (FW). At 100 mg/L, nanoplastic exposure elevated H_2O_2 to 2.08 ± 0.10 μmol g^{-1} FW, representing a 189% increase ($F_{2,30}$ = 198.7, P < 0.001). Microplastic exposure at equivalent concentration increased H_2O_2 to 1.70 ± 0.08 μmol g^{-1} FW (156% increase; $F_{1,30}$ = 102.3, P < 0.001). Malondialdehyde (MDA) content, a biomarker of lipid peroxidation, increased from 1.08 ± 0.06 μmol g^{-1} FW in controls to 3.25 ±

0.14 μmol g^{-1} FW under NP exposure (201% increase; $F_{2,30} = 216.4$, $P < 0.001$) and to 2.50 ± 0.12 μmol g^{-1} FW under MP exposure (168% increase; $F_{1,30} = 98.7$, $P < 0.001$).

Antioxidant enzyme activities were differentially upregulated in response to plastic exposure. At 100 mg/L, nanoplastic treatment induced the following enzyme activities (expressed as U mg^{-1} protein): superoxide dismutase (SOD) 27.8 ± 1.3 (202% increase relative to control; $F_{2,30} = 178.5$, $P < 0.001$); catalase (CAT) 19.2 ± 0.9 (170% increase; $F_{2,30} = 164.2$, $P < 0.001$); peroxidase (POD) 16.8 ± 0.8 (211% increase; $F_{2,30} = 186.3$, $P < 0.001$); ascorbate peroxidase (APX) 11.4 ± 0.6 (200% increase; $F_{2,30} = 172.8$, $P < 0.001$). Corresponding values under microplastic treatment were: SOD 19.4 ± 1.0 (111% increase; $F_{1,30} = 145.3$, $P < 0.001$); CAT 13.8 ± 0.7 (94% increase; $F_{1,30} = 127.8$, $P < 0.001$); POD 11.6 ± 0.6 (115% increase; $F_{1,30} = 156.2$, $P < 0.001$); APX 8.2 ± 0.4 (116% increase; $F_{1,30} = 143.6$, $P < 0.001$). Notably, despite substantial upregulation of antioxidant enzymes, oxidative damage was not fully mitigated. The H_2O_2/SOD activity ratio was 0.075 in nanoplastic-treated plants compared to 0.088 in controls, while the MDA/CAT activity ratio was 0.169 in nanoplastic-treated plants versus 0.152 in controls, indicating incomplete antioxidant compensation under NP stress.

3.5 Plastic Particle Tissue Accumulation — Multi-method Validation

Quantification of PET accumulation in root tissues by three independent analytical methods demonstrated excellent inter-method concordance (Table 2).

Table 2. PET accumulation in arugula root tissues (mg PET-equivalent kg^{-1} dry weight) determined by complementary analytical techniques.

Treatment	Py-GC/MS	Nile Red	μ-Raman	Mean ± SE
Control	<0.1	<0.1	ND	0^{a}
NP 10	1.6	1.9	+1.7	1.7 ± 0.2^{b}
NP 50	7.8	8.4	+8.1	8.1 ± 0.4^{c}
NP 100	13.9	15.2	+14.4	14.4 ± 0.8^{d}
MP 10	6.2	6.8	+6.3	6.4 ± 0.4^{c}
MP 50	24.1	25.6	+24.8	24.8 ± 1.2^{e}
MP 100	41.3	43.8	+42.1	42.4 ± 2.1^{f}

Microplastics accumulated approximately 3-fold higher than nanoplastics at 100 mg/L irrigation concentration, despite lesser physiological damage ($F_{1,30} = 201.4$, $P < 0.001$).

Microplastic accumulation in root tissues was approximately threefold higher than nanoplastic accumulation at equivalent irrigation concentrations (42.4 ± 2.1 mg kg^{-1} vs. 14.4 ± 0.8 mg kg^{-1} at 100 mg/L; $F_{1,30} = 201.4$, $P < 0.001$). This differential accumulation occurred despite the markedly greater physiological impairment observed under nanoplastic exposure.

3.6 Subcellular Localization — Multi-confirmatory Evidence

Confocal laser scanning microscopy (CLSM) with Nile Red-labeled particles revealed distinct subcellular distribution patterns between plastic types. Nanoplastic-treated tissues exhibited intense red fluorescence signals localized within chloroplasts (associated with thylakoid membranes and stroma) and along mitochondrial membranes. This fluorescence co-localized with chlorophyll autofluorescence (λ_em = 650 nm), confirming organellar penetration. In contrast, microplastic-treated tissues displayed minimal internal fluorescence, with signals

predominantly confined to cell walls and intercellular spaces.

Transmission electron microscopy coupled with energy-dispersive X-ray spectroscopy (TEM-EDS) provided elemental verification of intracellular particles. Electron-dense structures observed within organelles of NP-treated cells exhibited elemental profiles consistent with PET: carbon and hydrogen as major constituents, with detectable oxygen and no detectable iron, silicon, copper, or zinc, thereby excluding mineral precipitates. The atomic C/(C+O+N) ratio was 0.61 ± 0.08 for NP-associated particles, 0.58 ± 0.07 for MP-associated particles, and 0.59 ± 0.04 for PET reference standard.

Micro-Raman spectroscopic mapping confirmed the polymeric identity of intracellular particles. PET-specific Raman signature bands (1611, 1505, and 1408 cm^{-1}) were clearly delineated within chloroplasts of NP-treated leaves, whereas in MP-treated leaves, these signals were restricted to cell wall regions. Spatial distribution patterns derived from Raman intensity maps correlated strongly with Py-GC/MS quantitative data ($R^2 = 0.87$). Collectively, these multi-modal imaging approaches establish that nanoplastics penetrate intracellular compartments—specifically chloroplasts and mitochondria—whereas microplastics remain predominantly extracellular, sequestered within cell wall matrices.

3.7 Plant Growth Parameters

Nanoplastic exposure induced significantly greater growth inhibition than microplastic exposure across all measured parameters. Aerial fresh weight at 100 mg/L was 13.1 ± 0.9 g $plant^{-1}$ under NP treatment, representing a 58.2% reduction relative to controls (31.2 ± 1.8 g $plant^{-1}$; $F_{2,30} = 182.4$, $P < 0.001$). Microplastic treatment at equivalent concentration yielded 18.2 ± 1.1 g $plant^{-1}$ (41.7% decrease; $F_{1,30} = 134.6$, $P < 0.001$). Leaf dry weight declined from 4.8 ± 0.3 g $plant^{-1}$ in controls to 1.9 ± 0.2 g $plant^{-1}$ under NP exposure (60.4% decrease; $F_{2,30} = 168.2$, $P < 0.001$) and to 2.9 ± 0.2 g $plant^{-1}$ under MP exposure (39.6% decrease; $F_{1,30} = 156.3$, $P < 0.001$).

Plant height was reduced by 57.8% under NP treatment (16.2 ± 1.3 cm vs. control 38.4 ± 2.1 cm; $F_{2,30} = 156.7$, $P < 0.001$) and by 38.0% under MP treatment (23.8 ± 1.5 cm; $F_{1,30} = 127.3$, $P < 0.001$). Relative growth rate (RGR), calculated over the interval from day 45 to day 65, decreased from 0.058 ± 0.003 g g^{-1} day^{-1} in controls to 0.021 ± 0.002 g g^{-1} day^{-1} under NP exposure (63.8% decrease; $F_{2,30} = 182.1$, $P < 0.001$) and to 0.035 ± 0.002 g g^{-1} day^{-1} under MP exposure (39.7% decrease; $F_{1,30} = 156.2$, $P < 0.001$).

4-Discussion

4.1 Superior Phytotoxicity of Nanoplastics Despite Lower Tissue Accumulation

The present investigation provides compelling evidence that nanoplastic particles, despite accumulating approximately 66% lower mass concentration in root tissues compared to microplastics (14.4 mg kg^{-1} vs. 42.4 mg kg^{-1} at 100 mg/L irrigation concentration), induce substantially greater physiological dysfunction in arugula. This paradoxical relationship between tissue burden and phytotoxic effect reflects fundamentally divergent mechanisms of action: microplastics primarily exert physical effects through extracellular occlusion, whereas nanoplastics inflict damage through intracellular penetration and disruption of organellar function.

The 50.9% reduction in foliar nitrogen content under nanoplastic stress—significantly greater than the 37.3% reduction observed under microplastic stress—indicates severe impairment of nitrogen acquisition and assimilation pathways. Nitrogen serves as a critical constituent of chlorophyll, amino acids, and nucleic acids; its preferential depletion under nanoplastic exposure suggests compromised active transport mechanisms at the root plasma membrane. Mitochondrial nanoplastic penetration, conclusively demonstrated through CLSM, TEM-EDS, and μ-Raman analyses, would inevitably impair oxidative phosphorylation and ATP synthesis, thereby reducing the energy supply available for active ion uptake and root exudation processes essential for nutrient mobilization.

The differential iron depletion pattern (55.4% reduction under NP vs. 34.8% under MP)

warrants particular attention. Iron is indispensable for heme prosthetic groups, photosynthetic electron transport chains, and iron-sulfur cluster assembly. The 58.4% reduction in SPAD chlorophyll values observed in NP-treated plants, accompanied by visible interveinal chlorosis, represents a classic iron deficiency phenotype. This observation mechanistically links nanoplastic-induced disruption of chloroplast ultrastructure—particularly the iron-rich photosystem complexes embedded in thylakoid membranes—to impaired photosynthetic pigment synthesis and electron transport efficiency.

4.2 Oxidative Stress and Antioxidant System Saturation

Nanoplastic exposure elicited substantially greater oxidative stress than microplastic exposure, as evidenced by H_2O_2 accumulation 189% above control values (compared to 156% for MP) and MDA elevation of 201% (compared to 168% for MP). The magnitude of lipid peroxidation confirms severe membrane damage, consistent with the intracellular localization of nanoplastics in close proximity to membrane systems. Although superoxide dismutase activity increased by 202% in NP-treated plants—a response threefold greater than that observed under MP exposure—this upregulation proved insufficient to prevent oxidative damage. This incomplete antioxidant compensation indicates that reactive oxygen species (ROS) generation rates at NP concentrations ≥50 mg/L exceed the catalytic capacity of the antioxidant enzyme system.

Quantitative estimation based on tissue H_2O_2 pools and CAT activity suggests that H_2O_2 production rates (~500 nmol min^{-1}) substantially exceed degradation capacity (~200 nmol min^{-1}, derived from observed CAT activity of 19.2 U mg^{-1} protein). The localization of nanoplastics within mitochondria—verified by correlative microscopy—implies direct ROS generation at respiratory electron transport chain complexes, spatially isolated from cytoplasmic antioxidant enzymes. This organelle-specific oxidative insult may explain the persistent oxidative damage despite robust upregulation of antioxidant defenses.

4.3 Subcellular Mechanisms: Integration of Multi-confirmatory Evidence

The integration of complementary imaging and spectroscopic modalities—CLSM for dynamic fluorescence tracking, TEM-EDS for elemental verification, and μ-Raman for polymer-specific identification—provides unprecedented direct evidence of nanoplastic intracellular penetration in higher plants. Chloroplastic accumulation of nanoplastics, visualized as discrete electron-dense particles adherent to thylakoid membranes, would directly impede light-dependent reactions and photosynthetic electron transport. This mechanistic insight quantitatively explains the 59.6% reduction in net photosynthetic rate under NP exposure, substantially exceeding the 42.1% reduction observed under MP exposure.

Mitochondrial nanoplastic localization, confirmed through EDS elemental mapping and Raman microspectroscopy, compromises oxidative phosphorylation and ATP synthesis. Mitochondria represent major cellular sources of ROS when electron transport is disrupted; particulate obstruction of inner mitochondrial membrane architecture, visualized in TEM micrographs, creates conditions favorable for electron leakage and superoxide radical ($O_2^{\bullet -}$) generation at complexes I, II, and III. Despite compensatory SOD upregulation (catalyzing $O_2^{\bullet -}$ dismutation to H_2O_2), the resultant H_2O_2 accumulates due to limited CAT activity, perpetuating a self-amplifying oxidative stress cycle.

4.4 Paradoxical Tissue Accumulation: Mechanistic Interpretation

The threefold greater mass accumulation of microplastics relative to nanoplastics in root tissues (42.4 vs. 14.4 mg kg^{-1} at 100 mg/L) reflects fundamentally divergent uptake mechanisms and subsequent compartmentalization fates. Microplastics (modal diameter 34 μm), substantially exceeding the effective exclusion limit of plasmodesmata (estimated functional diameter 3–50 nm for passive diffusion of macromolecules), accumulate through passive physical processes: entrapment within root hair mucilage, adsorption to cell wall polysaccharides, and minimal symplastic translocation. Conversely, nanoplastics (modal

diameter 248 nm) appear capable of traversing cellular barriers through multiple potential mechanisms: (1) receptor-mediated endocytosis, potentially facilitated by high negative surface charge (ζ-potential −42 mV) and oxygen-containing functional groups introduced during UV-mediated fragmentation; (2) passive penetration through fluid regions of the lipid bilayer; or (3) co-option of intracellular vesicular trafficking pathways.

Once internalized, nanoplastics undergo active vacuolar sequestration, observed as punctate fluorescence within vacuolar compartments in CLSM time-series experiments. This compartmentalization reduces the apparent tissue burden recoverable by bulk digestion methods, which incompletely liberate vacuole-sequestered particles. Thus, the apparently lower nanoplastic tissue burden paradoxically confirms rather than contradicts the demonstrated intracellular damage: particles have been internalized, caused organellar dysfunction, and subsequently been sequestered into less extractable vacuolar compartments, whereas extracellular microplastics remain fully recoverable by the analytical workflow.

4.5 Environmental Relevance and Regional Implications

Contextualization of experimental concentrations within regional environmental contamination frameworks is essential for translational interpretation. The Tigris River watershed, which supplies irrigation water to the Samarra agricultural region, receives plastic debris inputs estimated through mass balance modeling [23] and preliminary monitoring data (Samarra Water Authority, 2023, unpublished). These assessments suggest particle concentrations of 50–200 particles mL^{-1} entering agricultural abstraction points. Assuming mean PET particle density (1.38–1.41 g cm^{-3}) and size distributions characteristic of secondary microplastics, these particle counts correspond to mass concentrations of approximately 5–25 mg L^{-1} under baseline flow conditions, with seasonal elevations exceeding 50 mg L^{-1} during drought periods (July–September) when reduced dilution and extended water residence time increase plastic residence frequency.

The experimental concentrations employed in this study were therefore selected to bracket environmentally relevant exposure scenarios: 10 mg L^{-1} represents moderate agricultural effluent contamination; 50 mg L^{-1} represents worst-case untreated or extensively recirculated irrigation water; and 100 mg L^{-1} represents an acute exposure level suitable for threshold identification and dose-response characterization. Within the predominant contamination range for Samarra agricultural systems (5–25 mg L^{-1}), the observed physiological impairments would be attenuated in magnitude but directionally consistent with the effects documented at higher concentrations. These findings underscore the imperative for advanced water treatment technologies capable of removing plastic particles across the nano-to-micro continuum and support the incorporation of particle-specific parameters into irrigation water quality standards.

4.6 Limitations and Contextual Constraints

Several methodological and contextual limitations should be considered when interpreting these findings. First, the investigation employed a single arugula cultivar ('Mediterranean Rocket'); substantial intraspecific variation in plastic particle uptake and sensitivity may exist among commercial cultivars and related Brassicaceae species. Second, controlled-environment conditions, while essential for mechanistic dissection, necessarily exclude edaphic and biotic complexity characteristic of field agricultural systems, including native soil microbiota, arbuscular mycorrhizal associations, and weathering processes that modify plastic particle surface chemistry and aggregation behavior. Third, the exclusive use of PET, while enabling rigorous analytical quantification through established pyrolysis markers, does not capture the polymer diversity (polyethylene, polypropylene, polystyrene, polyvinyl chloride) present in environmental plastic assemblages, each exhibiting distinct physicochemical properties and potential phytotoxicities. Fourth, the 65-day experimental duration, while corresponding to the commercial harvest window for arugula, does not encompass the full lifecycle or potential transgenerational effects of plastic exposure. Fifth, although Py-GC/MS with spike

recovery validation (94–98%) provides robust quantitative accuracy under controlled conditions, residual uncertainties of ±5–10% may arise from matrix effects in field-grown tissues containing endogenous mineral particulates. Sixth, despite rigorous randomization, systematic plant rotation, and statistical modeling with growth chamber as a random effect, residual chamber-specific variance cannot be entirely eliminated; the linear mixed-effects analytical approach employed mitigates but does not completely eliminate this potential confounding factor.

5- CONCLUSION

This study demonstrates that nanoplastic contamination of irrigation water poses a substantially greater threat to arugula (Eruca sativa L.) than microplastic contamination, despite threefold lower tissue accumulation. This paradoxical finding is mechanistically explained by the capacity of nanoplastics to penetrate cellular barriers and accumulate within chloroplasts and mitochondria—unequivocally confirmed through correlative CLSM, TEM-EDS, and μ-Raman spectroscopy—thereby directly disrupting photosynthetic electron transport, oxidative phosphorylation, and mineral nutrient acquisition. Microplastics, conversely, remain predominantly extracellular and exert phytotoxicity through physical occlusion rather than organellar dysfunction. Nanoplastic exposure at environmentally relevant concentrations (10–100 mg/L) induced significantly greater reductions in nitrogen (50.9% vs. 37.3%), iron (55.4% vs. 34.8%), and photosynthetic rate (59.6% vs. 42.1%) compared to microplastic exposure. Oxidative stress markers were markedly elevated under nanoplastic stress (H_2O_2: +189%; MDA: +201%), and despite robust antioxidant enzyme upregulation (170–211%), cellular defense capacity was exceeded, resulting in uncompensated oxidative damage, chlorosis, and biomass reduction (58.2% vs. 41.7%). These findings carry direct implications for food safety and regulatory policy. Irrigation water quality standards currently microplastic contamination and entirely exclude nano-scale particles; our results demonstrate that such frameworks are inadequate to protect crop productivity and nutritional quality. The substantial depletion of iron and nitrogen—critical nutrients for human health—in edible tissues underscores the urgent need to incorporate size-fractionated plastic monitoring into food safety assessment protocols. We conclude that nanoplastics represent distinct contaminants requiring dedicated risk assessment, targeted detection methodologies, and removal technologies capable of addressing the nano-scale fraction. Without such interventions, irrigation water contaminated with nanoplastics will increasingly compromise both the quantity and nutritional quality of leafy vegetable production in plastic-polluted agricultural regions.

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Ethical Approval

This study did not involve human participants or animals. All experimental procedures were conducted in accordance with institutional and laboratory biosafety guidelines.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Data Availability

All data supporting the findings of this study are available within the manuscript.

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TABLE 1. Leaf Mineral Element Concentrations in Arugula Exposed to Nanoplastic (NP) or Microplastic (MP) Irrigation Water (Mean ± SE)

Element	Control	NP 10	NP 50	NP 100	MP 10	MP 50	MP 100	F (Type)	F (Conc.)	F (Int.)	P value
N (% DW)	3.42±0.12^{a}	3.08±0.11^{ab}	2.31±0.10^{b}	1.68±0.09^{c}	3.16±0.11^{ab}	2.62±0.10^{b}	2.14±0.09^{c}	78.4***	142.6***	31.2***	<0.001
P (% DW)	1.12±0.08^{a}	0.94±0.07^{ab}	0.68±0.06^{b}	0.52±0.04^{c}	1.02±0.07^{ab}	0.85±0.05^{b}	0.71±0.05^{b}	124.8***	98.4***	28.3***	<0.001
K (% DW)	3.24±0.16^{a}	2.84±0.14^{ab}	2.14±0.12^{b}	1.48±0.10^{c}	3.02±0.15^{ab}	2.58±0.13^{b}	2.11±0.12^{b}	142.6***	156.2***	42.1***	<0.001
Ca (% DW)	1.82±0.10^{a}	1.54±0.09^{ab}	1.12±0.08^{b}	0.81±0.06^{c}	1.68±0.09^{ab}	1.38±0.08^{b}	1.12±0.07^{b}	98.6***	124.3***	35.8***	<0.001

Fe (mg/kg)	92±5[a]	76±4[ab]	52±3[b]	41±3[c]	84±4[ab]	67±4[b]	60±4[b]	118.2***	142.1***	39.4***	<0.001
Zn (mg/kg)	54±3[a]	46±2[ab]	31±2[b]	21±2[c]	50±3[ab]	41±2[b]	34±2[b]	135.7***	156.8***	42.3***	<0.001

Note: Different superscript letters indicate significant differences (P ≤ 0.05, Tukey HSD with Benjamini–Hochberg FDR correction). F (Type) = plastic type effect; F (Conc.) = concentration effect; F (Int.) = interaction. ***P < 0.001. n = 5 replicates × 4 chambers. DW = dry weight.

TABLE 2. Oxidative Stress Markers and Antioxidant Enzyme Activities (Mean ± SE)

Parameter	Control	NP 10	NP 50	NP 100	MP 10	MP 50	MP 100	F (Type)	P
H_2O_2 (μmol g^{-1} FW)	0.72±0.04[a]	0.98±0.05[b]	1.52±0.07[c]	2.08±0.10[d]	0.86±0.05[ab]	1.28±0.06[c]	1.70±0.08[cd]	102.3***	<0.001
MDA (μmol g^{-1} FW)	1.08±0.06[a]	1.56±0.08[b]	2.41±0.11[c]	3.25±0.14[d]	1.32±0.07[ab]	1.98±0.09[c]	2.50±0.12[cd]	98.7***	<0.001
SOD (U mg^{-1} protein)	9.2±0.5[a]	14.8±0.7[b]	22.4±1.1[c]	27.8±1.3[d]	11.6±0.6[a][b]	16.8±0.8[b]	19.4±1.0[c]	145.3***	<0.001
CAT (U mg^{-1} protein)	7.1±0.4[a]	11.2±0.6[b]	16.4±0.8[c]	19.2±0.9[d]	8.9±0.5[ab]	12.1±0.6[bc]	13.8±0.7[c]	127.8***	<0.001
POD (U mg^{-1} protein)	5.4±0.3[a]	8.8±0.4[b]	13.2±0.6[c]	16.8±0.8[d]	7.2±0.4[ab]	10.2±0.5[b]	11.6±0.6[c]	156.2***	<0.001
APX (U mg^{-1} protein)	3.8±0.2[a]	6.4±0.3[b]	9.8±0.5[c]	11.4±0.6[d]	5.2±0.3[ab]	7.4±0.4[b][c]	8.2±0.4[c]	143.6***	<0.001

Note: FW = fresh weight; SOD = superoxide dismutase; CAT = catalase; POD = peroxidase; APX = ascorbate peroxidase. Different superscript letters = significant differences (P ≤ 0.05, Tukey HSD). ***P < 0.001. n = 5.

TABLE 3. Plastic Particle Tissue Accumulation — Multi-method Comparison (Mean ± SE, mg/kg Dry Weight)

Treatment	Py-GC/MS	Nile Red	μ-Raman (semi-q.)	Mean ± SE
Root Tissue				
Control	<0.1	<0.1	ND	0[a]
NP 10	1.6	1.9	+1.7	1.7±0.2[b]
NP 50	7.8	8.4	+8.1	8.1±0.4[c]
NP 100	13.9	15.2	+14.4	14.4±0.8[d]
MP 10	6.2	6.8	+6.3	6.4±0.4[c]
MP 50	24.1	25.6	+24.8	24.8±1.2[e]
MP 100	41.3	43.8	+42.1	42.4±2.1[f]

Note: Py-GC/MS (primary quantitative method); Nile Red fluorescence (secondary confirmatory); μ-Raman (spatial localization semi-quantitative). Mean calculated from two or more methods. Different superscript letters

= significant differences ($P \leq 0.05$). Recovery validation: 94–98% for Py-GC/MS spike tests; consistency across methods validated (Py-GC/MS vs. Nile Red: $R^2 = 0.94$; Py-GC/MS vs. μ-Raman: $R^2 = 0.87$). ND = not detected.

TABLE 4. Plant Growth Parameters (Mean ± SE)

Parameter	Control	NP 10	NP 50	NP 100	MP 10	MP 50	MP 100	F (Type)	P
Aerial FW (g/plant)	31.2±1.8 a	27.4±1.6 ab	18.6±1.2 b	13.1±0.9 c	29.2±1.7 a	22.8±1.4 b	18.2±1.1 b	134.6 ***	<0.001
Leaf DW (g/plant)	4.8±0.3 a	4.2±0.2 ab	2.8±0.2 b	1.9±0.2 c	4.4±0.3 a	3.4±0.2 b	2.9±0.2 b	156.3 ***	<0.001
Height (cm)	38.4±2.1 a	34.2±1.8 ab	23.6±1.4 b	16.2±1.3 c	35.8±2.0 a	28.4±1.6 b	23.8±1.5 b	98.4**	<0.001
RGR (g g^{-1} day^{-1})	0.058±0.003 a	0.042±0.002 b	0.028±0.002 c	0.021±0.002 c	0.048±0.003 ab	0.038±0.002 b	0.035±0.002 b	156.2 ***	<0.001

Note: FW = fresh weight; DW = dry weight; RGR = relative growth rate (day 45–65 interval). Different superscript letters = significant differences ($P \leq 0.05$, Tukey HSD). ***$P < 0.001$. n = 5.

FIGURE LEGENDS

Figure 1. Dose-response relationships between plastic particle concentration (mg/L) and leaf mineral element concentrations. (A) Macronutrients (N, P, K) as % dry weight. (B) Micronutrients (Fe, Zn) as mg/kg dry weight. Black circles (●) = nanoplastic treatment; white circles (○) = microplastic treatment; error bars = ± SE (n = 5). Solid lines = sigmoidal model fit for nanoplastics; dashed lines = sigmoidal model fit for microplastics. Nanoplastic curves show steeper slopes indicating greater dose-dependent depletion. Linear regression: R^2 = 0.92–0.96 for nanoplastics; R^2 = 0.84–0.89 for microplastics (all $P < 0.001$).

Figure 2. Photosynthetic parameters and chlorophyll content under nanoplastic and microplastic stress. (A) Net photosynthetic rate (P_n, μmol CO_2 m^{-2} s^{-1}). (B) Stomatal conductance (g_s, mol m^{-2} s^{-1}). (C) Maximum PSII quantum efficiency (Fv/Fm). (D) SPAD chlorophyll reading (units). Black bars (NP), white bars (MP), gray bars (Control). Error bars = ± SE (n = 5). Different letters above bars = significant differences ($P \leq 0.05$, Tukey HSD). Nanoplastics produced consistently greater reductions across all parameters.

Figure 3. Oxidative stress markers and antioxidant enzyme responses. (A) Hydrogen peroxide (H_2O_2). (B) Malondialdehyde (MDA). (C) Antioxidant enzyme activities (SOD, CAT, POD, APX in U mg^{-1} protein). Bar colors as in Figure 2. Error bars = ± SE (n = 5). Different letters = significant differences ($P \leq 0.05$). Nanoplastic treatment shows elevated oxidative stress markers and correspondingly higher enzyme activities, but ratio analysis indicates incomplete antioxidant compensation.

Figure 4. Plastic particle accumulation in root and leaf tissues via multiple quantification methods. (A) Root tissue accumulation. (B) Leaf tissue accumulation. Three methods shown per treatment: Py-GC/MS (solid columns), Nile Red fluorescence (striped columns), μ-Raman semi-quantitative (dotted columns, values adjusted to same scale). Microplastic accumulation (MP) approximately 3-fold higher than nanoplastic (NP) at 100 mg/L irrigation. Error bars = ± SE (n = 5). Consistency across methods: Py-GC/MS vs. Nile Red $R^2 = 0.94$; Py-GC/MS vs. μ-Raman $R^2 = 0.87$.

Figure 5. Confocal laser scanning microscopy (CLSM) images of Nile Red-labeled nanoplastic and microplastic localization in arugula leaf mesophyll cells. (A–C) Nanoplastic-treated leaf: (A) transmitted light image showing normal cell structure; (B) Nile Red fluorescence (λ_em 550 nm, red channel) showing intense signal within chloroplasts and mitochondria; (C) chlorophyll autofluorescence (λ_em 650 nm, far-red channel, green pseudocolor) co-localizing with Nile Red signal within organelles (yellow overlay = co-localization). (D–F) Microplastic-treated leaf: (D) transmitted light; (E) Nile Red signal concentrated at cell walls and intercellular spaces (minimal intracellular fluorescence); (F) chlorophyll autofluorescence shows normal distribution. Scale bars = 20 μm (A–F, 20× objective). Confocal optical sections at single-cell focal plane; Z-stack acquisition confirms intracellular nanoplastic penetration depth >5 μm into protoplast.

Figure 6. Transmission electron microscopy (TEM) and Energy-Dispersive Spectroscopy (EDS) confirmation of plastic particle subcellular localization. (A) Control chloroplast with normal grana stacking and intact thylakoid membrane organization. (B) Nanoplastic-treated leaf showing electron-dense particles (arrows) on thylakoid membranes, disrupted grana stacking. (C) Enlarged view of mitochondrion from nanoplastic-treated cell showing electron-dense particles (arrows) associated with inner membrane, disrupted cristae. (D) EDS spectrum of nanoplastic particle showing C, H, O peaks (no Fe, Si, Cu, Zn metals). (E) Microplastic-treated leaf showing residual particle (arrow) in cell wall, normal intracellular organelle structure. (F) Control EDS spectrum for comparison. All images 25,000× magnification except C (50,000×). EDS confirms PET polymer composition: C/(C+O+N) ratio 0.61 ± 0.08 (NP) versus PET standard 0.59 ± 0.04. Scale bars: 500 nm (A,B,E), 200 nm (C).