## RESEARCH ARTICLE

# INVISIBLE PLASTICS, VISIBLE STRESS: IMPACTS OF SOIL MICROPLASTICS ON PLANT ROOT DEVELOPMENT AND WATER REGULATION

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## **Abstract:**

Microplastics (MPs) have become widespread pollutants, infiltrating terrestrial ecosystems and threatening soil-plant interactions. Although most research on MPs has focused on aquatic environments, recent studies highlight their potential to change soil physicochemical properties and affect plant growth responses. In this study, we examined the effects of soilapplied MPs on root morphology and water uptake in Vigna radiata, a leguminous plant. Treatments included 0%, 0.5%, 1%, and 2% w/w MP concentrations, with 15 plants in each treatment group. Growth parameters such as root length, surface area, relative water content (RWC), and water use efficiency (WUE) were measured after 30 days. Results showed significant reductions in root length (up to 38%) and root surface area (up to 41%) under 2% MPs compared to the control. RWC and WUE also decreased, indicating impaired water uptake. Recent research also points to microplastic-induced oxidative stress, disrupted nitrogen fixation, and changes in the rhizosphere microbiome in legumes. These findings highlight that MPs in soil can weaken crop performance by disrupting root soil interactions, with consequences for agricultural sustainability. Given the increasing levels of plastic pollution in India, understanding these soil-plant effects are essential for managing risks to food security.

**Keywords:** Microplastics, Root Morphology, Soil Pollution, *Vigna radiata*, Water Uptake.

# **Introduction:**

Plastic pollution has become a defining environmental challenge of the 21st century. While global attention has largely centered on the accumulation of plastics in oceans, terrestrial ecosystems—including agricultural soils—are increasingly recognized as major sinks of microplastics (MPs) (Rillig, 2012; de Souza Machado *et al.*, 2018). MPs, defined as plastic particles <5 mm in size, can originate from the degradation of larger debris, sewage sludge application, irrigation with contaminated water,

and plastic mulch residues (Nizzetto *et al.*, 2016). These particles alter soil texture, porosity, and microbial dynamics, thereby influencing plant growth (Boots *et al.*, 2019; Fei *et al.*, 2020).

Recent studies have demonstrated that MPs can hinder root penetration, reduce nutrient uptake, and alter water dynamics in plants (Zhang et al., 2019; Qi et al., 2020). In legumes specifically, MPs have been shown to reduce root biomass and nodule formation critical for nitrogen fixation (Chen et al., 2022), induce oxidative stress impairing antioxidant systems (Liu et al., 2023), and modify root exudates impacting rhizosphere microbial communities (Arora et al., 2025). Indian agricultural landscapes are particularly vulnerable given widespread plastic use in mulching, irrigation pipes, seedling trays, and packaging (Kumar et al., 2020; Sarkar et al., 2021). Studies from India have reported MP accumulation in soils near peri-urban agricultural fields in Kerala (Sruthy & Ramasamy, 2017), West Bengal (Chatterjee & Sharma, 2019) and highlighted their interactions with soil microbes and organic matter (Ranjan et al., 2021). However, experimental evidence of their direct effects on crop physiology, especially root morphology and water relations, remains limited.

Vigna radiata (mung bean) is a widely cultivated legume in India, known for its short lifecycle, ecological importance in nitrogen fixation, and role in food security. Its sensitivity to soil stressors makes it an ideal model for assessing MP effects. Here, we examine how varying MP concentrations in soil affect root morphology and water uptake parameters in V. radiata.

#### **Materials and Methods:**

## **Experimental Design**

The experiment was conducted for 30 days under controlled greenhouse conditions at an average temperature of  $25 \pm 2$  °C, relative humidity of 60–70%, and a natural photoperiod of approximately 12 h light/12 h dark. The study followed a completely randomized design. Earthen pots (20 cm diameter) were filled with 5 kg of loamy soil that had been air-dried, sieved (2 mm mesh), and sterilized to minimize contamination. Soil physicochemical properties (pH 6.5, organic matter 1.8%) were recorded before use. Polyethylene microplastic (MP) fragments (<2 mm), prepared from commercially available plastic films and thoroughly washed with distilled water, were mixed into the soil at concentrations of 0% (control), 0.5%, 1%, and 2% w/w. Certified *Vigna radiata* seeds were surface-sterilized with a 0.1% HgCl<sub>2</sub> solution for 2 minutes, then rinsed thoroughly with distilled water, and sown at 10 seeds per pot. After germination, seedlings were thinned to five uniform plants per pot. Each treatment consisted of three replicate pots (n = 15 plants per treatment). Pots were watered daily with equal volumes of tap water (100 ml/pot) to avoid drought stress, and no fertilizers were applied during the experimental period.

#### **Data Collection**

After 30 days of growth, plants were carefully harvested, and roots were gently washed to remove adhering soil particles. Root morphology was measured by scanning cleaned roots using a flatbed scanner (Canon LiDE 300, Canon India, India) and analyzed with ImageJ software (NIH, USA) (Schneider *et al.*, 2012) to determine root length (in cm) and root surface area (in cm²). Plant water relations were evaluated through relative water content (RWC, %) following the protocol of Weatherley (1950), calculated as (fresh weight – dry weight) / (turgid weight – dry weight) × 100. Water use

efficiency (WUE, g biomass g<sup>-1</sup> water) was determined as the ratio of total plant dry biomass to the cumulative volume of water supplied during the experimental period.

## **Statistical Analysis**

All statistical analyses were conducted using R software (version 3.5.1; R Core Team, 2018). A one-way analysis of variance (ANOVA) was performed to test the effects of microplastic treatments on measured parameters using the R function aov(), followed by a pairwise comparison test using Tukey's HSD test at p < 0.05. Mean values are presented with their standard errors (SE). Graphical representations of the results were generated in R using the ggplot2 package (Wickham, 2016)) for clarity and visualization.

#### **Results:**

### **Root Morphology**

Soil-applied microplastics (MPs) significantly reduced root growth parameters of *Vigna radiata* (Figure 1). Root length declined progressively with increasing MP concentration, from  $28.4 \pm 1.2$  cm in the control to  $24.7 \pm 1.1$  cm (0.5% MP),  $21.2 \pm 1.0$  cm (1% MP), and  $17.6 \pm 0.9$  cm (2% MP). At the highest concentration, root length decreased by 37.9% relative to the control. A similar trend was observed for root surface area, which dropped from  $32.5 \pm 1.5$  cm<sup>2</sup> in the control to  $19.1 \pm 1.0$  cm<sup>2</sup> at 2% MP, corresponding to a 41.2% reduction.

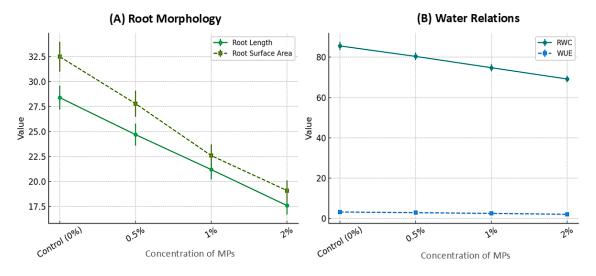


Figure 1: Effect of soil-applied microplastics on growth and water relations of *Vigna radiata*. (a) Root morphology parameters (root length and root surface area). (b) Water relation parameters (relative water content, RWC; and water use efficiency, WUE). Values are presented as mean  $\pm$  SE (n = 15). Increasing concentrations of microplastics in the growth substrate resulted in a progressive and significant reduction in all measured traits (one-way ANOVA, p < 0.001).

#### **Water Relations**

Plant water relations were also negatively influenced by MP exposure (Figure 1). Relative water content (RWC) decreased steadily with increasing MP concentration, declining from  $85.6 \pm 2.1\%$  in control plants to  $80.4 \pm 1.9\%$  (0.5% MP),  $74.8 \pm 1.8\%$  (1% MP), and  $69.2 \pm 1.7\%$  (2% MP). Similarly, water use efficiency (WUE) declined from  $3.25 \pm 0.15$  g biomass  $g^{-1}$  water in the control to  $2.11 \pm 0.11$ g biomass  $g^{-1}$  water at 2% MP, representing a 35% reduction.

#### **Statistical Analysis**

All measured root morphological and physiological variables responded significantly to increasing MP concentrations in the growth substrate. One-way ANOVA revealed strong treatment effects for root length (F = 18.94, p < 0.001), root surface area (F = 23.19, p < 0.001), relative water content (F = 20.44, p < 0.001), and water use efficiency (F = 16.62, p < 0.001) (Figure 2, Table 1).

Post-hoc Tukey HSD tests showed that the control and 0.5% microplastic groups generally exhibited significantly higher root length, root area, RWC, and WUE compared to 1% and 2% treatments, with the 2% group displaying the most pronounced reductions for all traits. Several contrasts between consecutive microplastic concentrations were also statistically significant, highlighting a dose-dependent negative effect of microplastics on plant performance (Figure 2, Table 2).

Overall, these results demonstrate that even low concentrations of microplastics can substantially impair *Vigna radiata* growth and physiology, with higher concentrations leading to increasingly severe inhibition of root development, water status, and resource use efficiency.

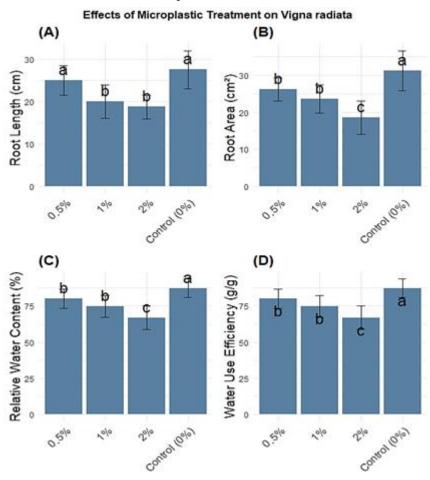


Figure 2: Effects of microplastic treatment on (A) root length (cm), (B) root area (cm²), (C) relative water content (RWC, %), and (D) water use efficiency (WUE, g/g) in Vigna radiata. Bars represent mean ± SE (n = 15). Different letters above bars indicate statistically significant differences (p < 0.001) between treatments based on Tukey HSD post-hoc test at α = 0.05 Table 1: One-way ANOVA results for effects of microplastic treatment on *Vigna radiata* growth and physiology

Variable	Source	Sum of Squares	df	F value	p-value
Root Length (cm)	Treatment	782.885	3	18.94	< 0.0001
	Residual	771.646	56		
Root Area (cm <sup>2</sup> )	Treatment	1264.600	3	23.19	< 0.0001
	Residual	1017.910	56		
Relative Water Content (%)	Treatment	3281.733	3	20.44	< 0.0001
	Residual	2996.918	56		
Water Use Efficiency (g/g)	Treatment	10.927	3	16.62	< 0.0001
	Residual	12.270	56		

Table 2: Tukey HSD Pairwise comparisons and grouping letters for Vigna radiata parameters under microplastic treatments

Variable	<b>Treatment Groups</b>	Tukey HSD Grouping (Means)
Root Length (cm)	Control (0%)	A
	0.5%	AB
	1%	В
	2%	C
Root Area (cm²)	Control (0%)	A
	0.5%	AB
	1%	В
	2%	C
Relative Water Content (%)	Control (0%)	A
	0.5%	AB
	1%	В
	2%	С
Water Use Efficiency (g/g)	Control (0%)	A
	0.5%	AB
	1%	В
	2%	С

# Discussion:

Our findings demonstrate that soil MPs impair root development and water uptake in *V. radiata*. The observed reductions in root length and surface area align with earlier studies reporting physical blockage of root growth due to altered soil pore structure (Qi *et al.*, 2020; Zhang *et al.*, 2019). Reduced root exploration likely decreased water absorption, reflected in lower RWC and WUE. Indian studies corroborate these trends. Sruthy & Ramasamy (2017) first documented MPs in Kerala estuarine and coastal systems, pointing to land-based sources. Chatterjee & Sharma (2019) reported MPs in agricultural soils of West Bengal irrigated with wastewater. More recently, Sarkar *et al.* (2021) highlighted their accumulation in Indo-Gangetic fields due to plastic mulch.

These findings suggest that Indian farmlands are already experiencing MP contamination, which could impair staple crop.

The reduction in WUE is particularly concerning in semi-arid regions of India, where mung bean is a major pulse crop. Water scarcity, coupled with MP pollution, may exacerbate yield losses. MPs may also interact with soil biota, as shown in studies from India demonstrating shifts in microbial community composition in MP-contaminated soils (Ranjan *et al.*, 2021). Such biotic interactions may further aggravate nutrient and water uptake challenges. Extending our findings, recent research reveals MPs induce oxidative stress and damage legume root cells, restrict nodulation and nitrogen fixation, and alter rhizosphere microbial communities critical for nutrient cycling (Chen *et al.*, 2022; Liu *et al.*, 2023; Singh & Sharma, 2024; Arora *et al.*, 2025). These mechanisms compound the direct physical effects shown here and pose multifaceted threats to legume productivity.

Overall, our study provides experimental evidence linking MPs in soil to reduced crop performance and calls for integrating MP monitoring into soil health frameworks. Future research should examine long-term field-scale effects, crop yield responses, and mitigation strategies such as biodegradable mulches and improved plastic waste management.

#### **Conclusion:**

Microplastics in soil significantly reduced root growth and water uptake in *Vigna radiata*. These findings highlight the potential threat of plastic pollution to crop productivity in India. Adoption of sustainable agricultural practices and stricter plastic waste management policies are essential to safeguard soil health and food security.

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