# EFFECT OF SILVER NANOPARTICLES AND MICROPLASTICS ON OXIDATIVE STRESS IN *Allium cepa* ROOTS

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## INTRODUCTION

Silver nanoparticles (AgNPs) are increasingly used in medicine, agriculture, and industry due to their strong antimicrobial activity and favorable physicochemical properties [1]. To prevent agglomeration, they are often coated with stabilizing agents such as polyvinylpyrrolidone (PVP) and cetyltrimethylammonium bromide (CTAB) [2]. However, their widespread application raises environmental concerns, as AgNPs can accumulate in ecosystems and enter food chains [3]. Microplastics (MPs), as persistent pollutants, can adsorb AgNPs, potentially altering their mobility, bioavailability, and toxicity in the environment [4]. Both AgNPs and MPs can be taken up by plants, either via root surface adhesion or vascular transport, posing risks to plant health. *Allium cepa* is a sensitive and widely accepted model for evaluating the phytotoxic effects of such contaminants, making it suitable for studying the combined effect of AgNPs and MPs on terrestrial plant systems [5].

## MATERIALS AND METHODS

To investigate AgNPs and MPs impact on terrestrial plants, *A. cepa* roots were exposed for 72 h to AgNPs coated with either PVP or CTAB at a concentration of 10.79 mg $L^{-1}$. Two types of MPs, polystyrene (PS-MPs) and polymethyl methacrylate (PMMA-MPs), were applied at 40 and 400 mg $L^{-1}$, both alone and in combination with AgNPs. To assess oxidative damage and stress response, lipid peroxidation level, hydrogen peroxide ($H_2O_2$) and protein carbonyl content were measured [6-8]. The activity of antioxidant enzymes, catalase (CAT), ascorbate peroxidase (APX), pyrogallol peroxidase (PPX), and superoxide dismutase (SOD), was also evaluated [9-11]. In addition, the uptake of AgNPs and MPs into root tissues was examined to determine internalization patterns after exposure.

## RESULTS

### OXIDATIVE STRESS PARAMETERS

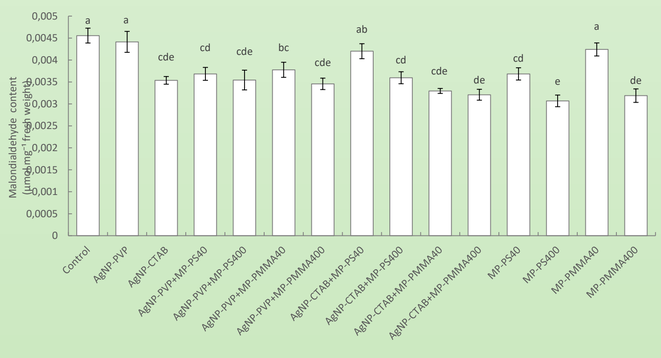


**Figure 1.** Malondialdehyde content in *A. cepa* roots after 72 h exposure to 10.79 mg $L^{-1}$ AgNP-PVP and AgNP-CTAB and 40 or 400 mg $L^{-1}$ MP-PS and MP-PMMA, applied individually or in combination. Values represent means ± standard error from two independent experiments, each with six replicates (n = 12). Treatments that differ significantly at $p \leq 0.05$ (one-way ANOVA followed by Duncan's post hoc test) are marked with different letters.

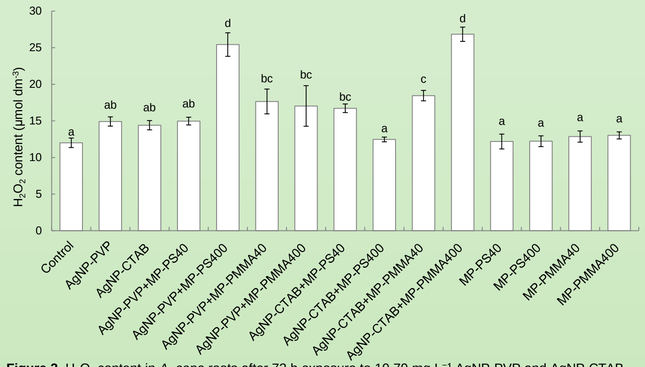


**Figure 2.** $H_2O_2$ content in *A. cepa* roots after 72 h exposure to 10.79 mg $L^{-1}$ AgNP-PVP and AgNP-CTAB and 40 or 400 mg $L^{-1}$ MP-PS and MP-PMMA, applied individually or in combination. Values represent means ± standard error from two independent experiments, each with six replicates (n = 12). Treatments that differ significantly at $p \leq 0.05$ (one-way ANOVA followed by Duncan's post hoc test) are marked with different letters.

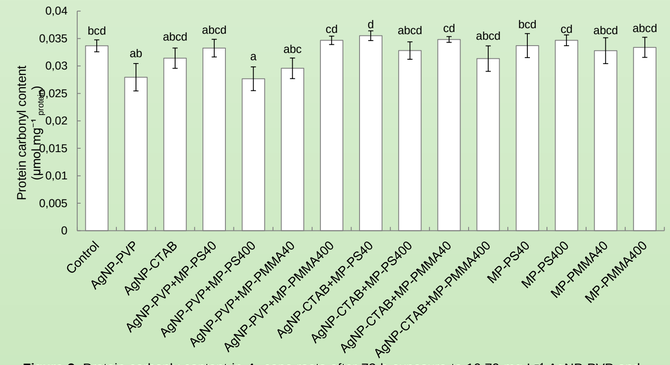


**Figure 3**. Protein carbonly content in *A. cepa* roots after 72 h exposure to 10.79 mg $L^{-1}$ AgNP-PVP and AgNP-CTAB and 40 or 400 mg $L^{-1}$ MP-PS and MP-PMMA, applied individually or in combination. Values represent means ± standard error from two independent experiments, each with six replicates (n = 12). Treatments that differ significantly at $p \leq 0.05$ (one-way ANOVA followed by Duncan's post hoc test) are marked with different letters.

### ACTIVITY OF ANTIOXIDANT ENZYMES

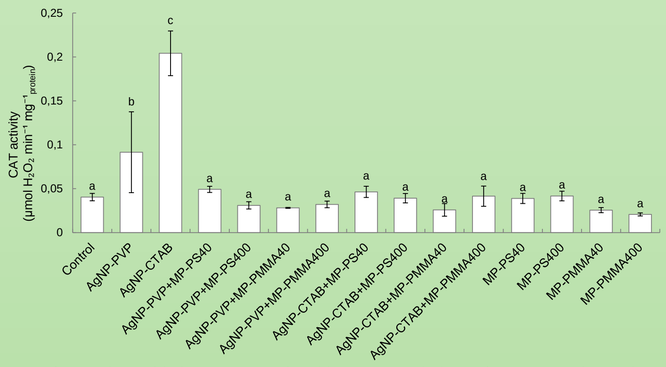


**Figure 4.** CAT activity in *A. cepa* roots after 72 h exposure to 10.79 mg $L^{-1}$ AgNP-PVP and AgNP-CTAB and 40 or 400 mg $L^{-1}$ MP-PS and MP-PMMA, applied individually or in combination. Values represent means ± standard error from two independent experiments, each with six replicates (n = 12). Treatments that differ significantly at $p \leq 0.05$ (one-way ANOVA followed by Duncan's post hoc test) are marked with different letters.

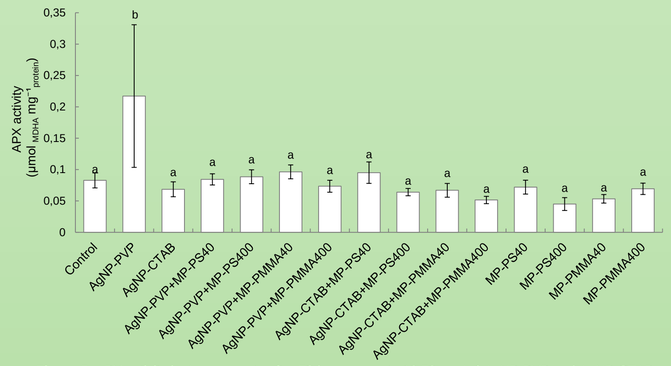


**Figure 5.** APX activity in *A. cepa* roots after 72 h exposure to 10.79 mg $L^{-1}$ AgNP-PVP and AgNP-CTAB and 40 or 400 mg $L^{-1}$ MP-PS and MP-PMMA, applied individually or in combination. Values represent means ± standard error from two independent experiments, each with six replicates (n = 12). Treatments that differ significantly at $p \leq 0.05$ (one-way ANOVA followed by Duncan's post hoc test) are marked with different letters.

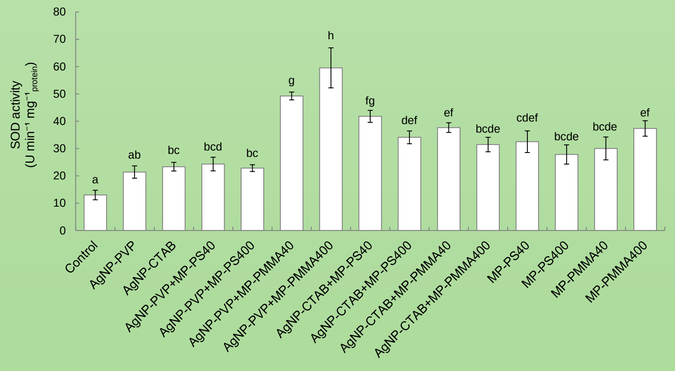


**Figure 6.** SOD activity in *A. cepa* roots after 72 h exposure to 10.79 mg $L^{-1}$ AgNP-PVP and AgNP-CTAB and 40 or 400 mg $L^{-1}$ MP-PS and MP-PMMA, applied individually or in combination. Values represent means ± standard error from two independent experiments, each with six replicates (n = 12). Treatments that differ significantly at $p \leq 0.05$ (one-way ANOVA followed by Duncan's post hoc test) are marked with different letters.

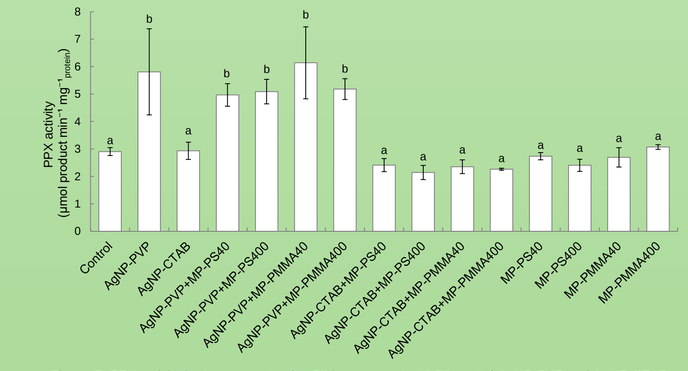


**Figure 7.** PPX activity in *A. cepa* roots after 72 h exposure to 10.79 mg $L^{-1}$ AgNP-PVP and AgNP-CTAB and 40 or 400 mg $L^{-1}$ MP-PS and MP-PMMA, applied individually or in combination. Values represent means ± standard error from two independent experiments, each with six replicates (n = 12). Treatments that differ significantly at $p \leq 0.05$ (one-way ANOVA followed by Duncan's post hoc test) are marked with different letters.

### AgNPs AND MPs UPTAKE

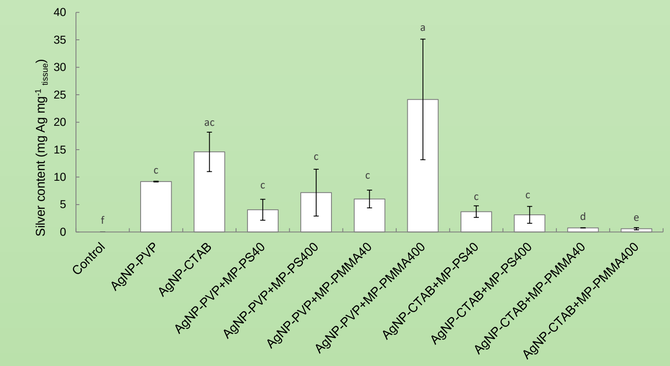


**Figure 8.** Silver uptake in *A. cepa* roots after 72 h exposure to 10.79 mg $L^{-1}$ AgNP-PVP and AgNP-CTAB alone or in combination with 40 or 400 mg $L^{-1}$ MP-PS and MP-PMMA. Values represent means ± standard error from one experiment with three replicates (n = 3). Treatments that differ significantly at $p \leq 0.05$ (one-way ANOVA followed by Duncan's post hoc test) are marked with different letters.

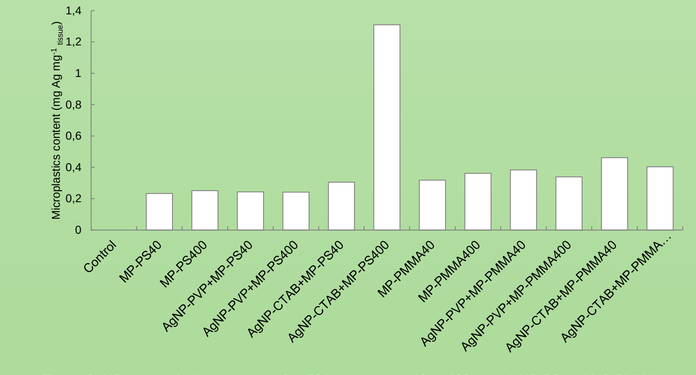


**Figure 9.** MPs uptake in *A. cepa* roots after 72 h exposure to 40 or 400 mg $L^{-1}$ MP-PS and MP-PMMA, alone or in combination with 10.79 mg $L^{-1}$ AgNP-PVP and AgNP-CTAB. Values represent one replicate.

## ACKNOWLEDGMENT

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## CONCLUSIONS

**1. Primary Oxidative Stress Driver**: AgNPs, not MPs, were the main source of oxidative stress, as indicated by elevated $H_2O_2$ levels. MPs enhanced this stress synergistically, particularly when combined with MP-PMMA and high AgNP concentrations.

**2. Oxidative Stress Without Biomolecular Damage**: Despite the rise in $H_2O_2$, no significant damage was observed in lipids and proteins, the key biomolecules, suggesting a controlled oxidative stress response.

**3. AgNP Coating-Dependent Enzymatic Response**: The surface coating of AgNPs played a key role in modulating antioxidant enzyme activity.

- AgNP-CTAB induced CAT activity, but this effect diminished with MPs presence due to reduced AgNP uptake.
- AgNP-PVP triggered strong responses in APX, PPX, and (SOD), especially when combined with MP-PMMA.

**4. AgNP Uptake Affected by MPs**: Generally, MPs reduced AgNP uptake, except in the AgNP-PVP and MP-PMMA high-concentration treatment, where uptake, and consequently enzyme activity, was enhanced.

**5. MP Uptake Unaffected by AgNPs**: The presence of AgNPs did not significantly influence MPs uptake.