

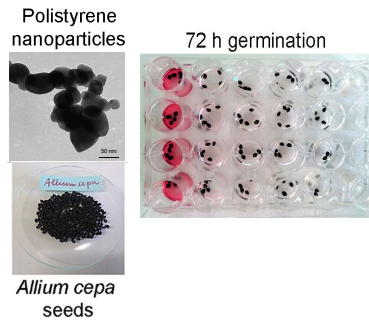
Toxicity induction by nano-polystyrene in *Allium cepa* L. seedlings

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CONTEXT

In recent years there has been a great emphasis on the problems related to worldwide plastic pollution which affects all environmental matrices such as marine and fresh water, soil and atmosphere. The main issue is that plastics persist in the environment, undergoing a slow process of fragmentation into small pieces generating microplastics and nanoplastics. Especially the smaller fractions are of specific concern as they have been found in agroecosystems, interacting with animals and plants.



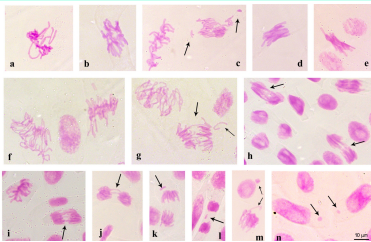
EXPERIMENTAL

It is very important to assess the environmental impact risks of nanoplastics through ecotoxicological tests. Phytotoxicity and genotoxicity studies, oxidative stress markers analysis and TEM observation were performed on *Allium cepa* L. seedlings.

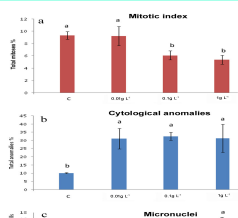
In this work, the effects of plastic nanoparticles (p-NPS) (Red Dyed Polystyrene, 50 nm) at 3 different concentrations (0.01, 0.1 and 1 g L⁻¹) were evaluated on *A. cepa* L. Acute phytotoxicity effects were observed during seed germination after 3 days exposure, while genotoxic effects were revealed by cytogenetic analysis of primary root meristems, in a dose dependent manner.

RESULTS

Genotoxicity

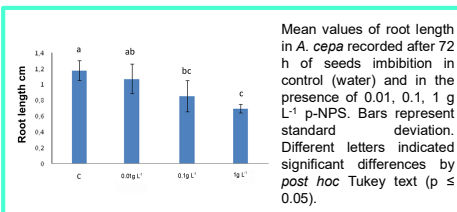


Cytogenetic analysis in *A. cepa* treated with different concentrations of p-NPS. Cytological anomalies (arrows): (a, b, c) abnormal c-metaphase (1 g L⁻¹); (d, e) sticky metaphases (0.01 and 0.1 g L⁻¹, respectively); (f, g) abnormal anaphase with lagging chromosomes and bridges (0.1 and 1 g L⁻¹, respectively); (h, i, j) sticky anaphases (h, 0.01 g L⁻¹; i, j, 1 g L⁻¹); (k, l) lagging chromosomes at anaphase (1 g L⁻¹); (m, n) lagging chromosome and micronuclei (0.1 and 1 g L⁻¹, respectively).



Results of cytological analysis of *A. cepa* root meristems after 72 h of seeds imbibition in control (water) and in the presence of 0.01, 0.1, 1 g L⁻¹ p-NPS. a) Mean values of Mitotic index; b) % of total cytological abnormalities (abnormal metaphases + abnormal ana/telophases); c) micronuclei frequency (%). Bars represent standard deviation. Different letters indicated significant differences by *post hoc* Tukey text ($p \leq 0.05$).

Phytotoxicity



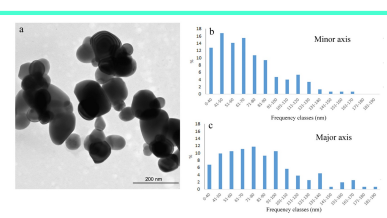
Mean values of root length in *A. cepa* recorded after 72 h of seeds imbibition in control (water) and in the presence of 0.01, 0.1, 1 g L⁻¹ p-NPS. Bars represent standard deviation. Different letters indicated significant differences by *post hoc* Tukey text ($p \leq 0.05$).

Oxidative stress marker analysis

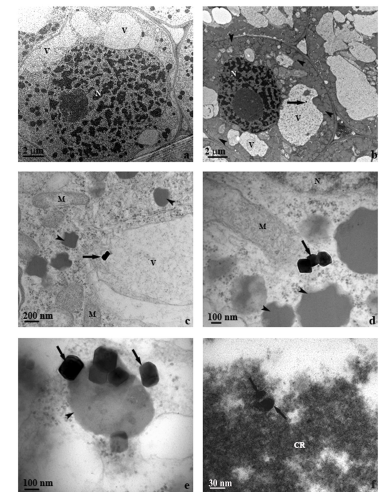
Table 1. Germination percentage, hydrogen peroxide (H₂O₂) and thiobarbituric acid reactive substances (TBARS) contents in roots from seeds of *A. cepa* treated for 72 h with water (C), 0.01, 0.1 and 1.0 g L⁻¹ p-NPS. Values are means of at least four replicates \pm SD. Different letters denote significant differences at $p < 0.05$.

Parameters	C	0.01 g L ⁻¹	0.1 g L ⁻¹	1.0 g L ⁻¹
Germination %	100.0 \pm 0.0a	98.75 \pm 2.5a	97.5 \pm 2.8a	96.25 \pm 2.5a
H ₂ O ₂ (nmol g ⁻¹ FW)	0.41 \pm 0.05b	0.30 \pm 0.07b	0.55 \pm 0.04b	1.03 \pm 0.31a
TBARS (nmol g ⁻¹ FW)	25.35 \pm 0.9b	22.04 \pm 0.6c	26.38 \pm 1.1b	30.12 \pm 1.9a

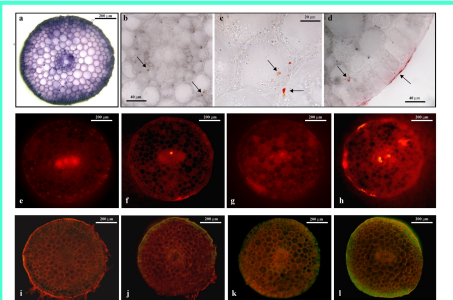
TEM analysis



Size characterization of p-NPS: a) TEM observation; size distribution of particles grouped in frequency classes for b) minor and c) major axis, after ImageJ program elaboration of TEM images.



TEM images of cell portion of *A. cepa*. a) control root; b) 1 g L⁻¹ p-NPS treated root; c and d) 0.1 g L⁻¹ p-NPS treated root; e and f) 1 g L⁻¹ p-NPS treated root. The arrows indicate nanoparticles, the arrow heads indicate electron dense bodies. CR, chromatin; M, mitochondria; N, nucleus; V, vacuole.



Cross hand sections of *A. cepa* roots of seedlings after 72 h germination in control (water), and in the presence of p-NPS. The plate comprehends representative images of: toluidine blue stained root section (control) (a), and some details of the root compartments, in treated samples, showing central cylinder (b), cortical cylinder (c-d) and root epidermis (d). In b, c and d red spots represent p-NPS aggregates in 1 g L⁻¹ treatment (arrows); *in situ* detection of H₂O₂ by Amplex UltraRed Reagent in control (e) and in samples treated with 0.01, 0.1 and 1 g L⁻¹ p-NPS (f, g, h); *in situ* detection by BODIPY reagent of lipid peroxidation in control (i) and in samples treated with 0.01, 0.1 and 1 g L⁻¹ p-NPS (j, k, l).

Table 2. Cytological analysis of *A. cepa* root meristems in control and after 72 h treatments with 0.01, 0.1, 1, g L⁻¹ p-NPS. Normal and abnormal mitotic phases (prophases, metaphases and ana/telophases) were expressed as mean values \pm standard deviation on 100 mitoses analyzed. Values are means of at least four replicates \pm SD. Different letters denote significant differences at $p < 0.05$.

	C	0.01 g L ⁻¹	0.1 g L ⁻¹	1.0 g L ⁻¹
%Prophases	39.6 \pm 1.9a	44.3 \pm 8.3a	40.8 \pm 6.9a	32.7 \pm 5.3a
%Metaphases	16.5 \pm 4a	10.06 \pm 5.8a	9.07 \pm 4.3a	9.21 \pm 4.1a
%Abn Metaphases	8.6 \pm 4.1b	18.49 \pm 2.9a	17.5 \pm 3.9a	14.65 \pm 3.7ab
%Ana/telophases	27.8 \pm 2.4a	20.76 \pm 5.5a	17.5 \pm 4.8a	26.7 \pm 12a
%Abn Ana/telophases	7.5 \pm 1.9b	12.7 \pm 3.5ab	15.1 \pm 4.8ab	16.7 \pm 5.5a

CONCLUSIONS

- Our data indicate that plants can be damaged by exposure to p-NPS in terms of phytotoxicity, genotoxicity and oxidative stress induction and that p-NPS can enter plant cells.
- Phytotoxicity results (seed germination and root length endpoints) indicated acute effects of p-NPS in *A. cepa*; cytogenetic data (mitotic index, cytogenetic anomalies and micronuclei) indicated a dose-dependent genotoxic effect. The oxidative stress markers analysis (content of H₂O₂ and lipid peroxidation) highlighted toxic effects especially at the highest doses. TEM analysis of root cells allowed the evaluation of p-NPS internalization and damages in different cell compartments.
- Considering the ability of the analysed crop to internalize p-NPS, their presence in plant tissues can induce contamination of food plants with entry into the food chain.
- The pollution of agricultural land by plastics, therefore, is to be carefully monitored.