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Fate and effect of tire rubber ash nano-particles (RANPs) in cucumber

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ABSTRACT

There are growing interests on effects of nano-materials on living organisms including higher plants. No report is available on positive and negative effects of rubber ash nano-particles (RANPs) on edible plants. Recently, we reported the possibility of using waste tire rubber and rubber ash as zinc (Zn) fertilizer for plants. In this nutrient solution culture study, for the first time, root uptake and the effects of RANPs on growth and Zn, cadmium (Cd), and lead (Pb) concentration in cucumber was investigated. Various Zn levels (0, 1, 5, 25, 125 mg L^{-1}) were applied in the form of RANPs or $ZnSO_4$. The root RANPs uptake was visualized by light (LA), scanning electron (SEM), and transmission electron microcopies (TEM). At all Zn levels, cucumber plants supplied with RANPs produced higher shoot and root biomass compared with those supplied with $ZnSO_4$. In addition, the RANPs resulted in higher accumulation of Zn in cucumber tissues in comparison with $ZnSO_4$; although phytotoxicity of Zn from $ZnSO_4$ was greater than that from RANPs. Clear evidence of the RANPs penetration into the root cells was obtained by using SEM and TEM. Filaments of RANPs were also observed at the end of roots by LM and TEM. Further research is needed to clarify the fate of the RANPs in plant cells and their possible risks for food chain.

1. Introduction

There are growing concerns and interests on the effects of nano-materials on living organisms, including higher plants. Nano-particles can greatly change the physicochemical properties of the materials and may adversely affect growth and yield of higher plants. Limited information is available on the effects of nano-particles on higher plants. Both positive and negative effects of nano-particles on seed germination, root growth and plant yield have been reported (Lei et al., 2007; Lin and Xing, 2007; Torney et al., 2007). The effect of nano-particles on plant is dependent on their properties, dose and method of application, plant species and surrounding solution conditions (Lin and Xing, 2008). A positive effect of titanium (Ti) nano-particles at optimum concentration on photosynthesis, nitrogen metabolism, and growth of spinach has been reported (Hong et al., 2005; Yang et al., 2007). Nano-particles of Al had no significant effect on growth of California red kidney bean and ryegrass (Doshi et al., 2008; Lin and Xing, 2008) while they inhibited root growth of corn, soybean, cabbage, cucumber, and carrot (Yang and Watts, 2005). Severe detrimental effects of ZnO nano-particles have also been reported on root elongation of ryegrass, radish, and rape (Lin and Xing, 2007). ZnO nano-particles also negatively affected root epidermal and cortical cells of ryegrass (*Lolium perenne* L.) (Lin and Xing, 2007). Nano-particles of ZnO strongly adhered onto the ryegrass root surfaces; although individual ZnO nano-particles were observed by scanning electron and transmission electron microscopy in apoplast and protoplast of the root endodermal and xylem cells (Lin and Xing, 2007). Translocation of ZnO nano-particles from root to shoots of ryegrass has also been reported (Carpita et al., 1979; Lin and Xing, 2007; Remya et al., 2010).

Recently, we reported the possibility of using waste tire rubber and rubber ash as Zn fertilizer for plants (Taheri et al., 2011). Tire rubber ash is rich in zinc (~10%) and might be applied as an effective and safe fertilizer source for supplying this nutrient element with no risk of Cd contamination (Chaney, 2007; Taheri et al., 2011). Tire rubber ash was a very effective amendment to increase the diethylene triamine pentaacetic acid (DTPA)-extractable Zn in a calcareous Zn-deficient soil (Chaney, 2007). Zinc applied in rubber ash is a relatively rapidly released source of Zn in soil, showing results similar to laboratory grade Zn-sulfate in increasing the DTPA-extractable soil Zn (Chaney, 2007). According to the previous results, rubber particles size is a key factor determining the rate of micronutrient release (Chaney, 2007). Tire rubber ash contains particles at nano-sizes ($<$ 100 nm) with high solubility in water (Milbocker, 1974). High specific surface of nano-

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particles increase the availability of element for plant (Remya et al., 2010). It is hypothesized that nano-fertilizers are rapidly and completely absorbed by plant tissue (Yang et al., 2006). On the other hand, phytotoxicity of nano-particles might be an important limiting factor for their application in agriculture. Despite increase of knowledge on phytotoxicity of nano-particles, the phytotoxicity mechanism remains unknown and limited information is available on the potential uptake of nano-particles by plants. No report is available on the fate of rubber ash nano-particles (RANPs) in edible plants. In this study, for the first time, we investigated the root uptake of RANPs and their effects on growth and Zn, Cd, and Pb uptake by cucumber plants grown in nutrient solution.

2. Materials and methods

2.1. Tire rubber ash nano-particles

Samples of tire rubber debris were obtained from the Rubber Industry of Yazd Tire in Iran. After separating the metal wire of scrap tire, they were reduced to <10-cm shreds by a slow speed shredder. The shreds went through three successively narrower blade shredders to further reduce shreds to <6-cm. The particles were processed to smaller sizes by grinding rolling mills and sieving them on a stainless steel sieve (<1 mm screen). Ground tire rubber was ashed in a furnace at 550 °C for 12 h. The tire rubber ash sample was then milled at 500 rotations per min (rpm) for 5 h in a ball mill with ball to powder rate of 10:1 on a dry weight basis. The approximate size of particles after milling was measured qualitatively using scanning electron microscopy (SEM), dynamic light scattering (DLS), and transmission electron microcopies (TEM).

2.2. Characterization of nano-particles by SEM, DLS, and XRD

The tire rubber ash particles after milling were characterized by Philips XL30 Robeahi SEM. The tire rubber ash particles were coated by gold for 60 s and imaged by SEM. In addition, a sample of suspended particles in the nutrient solution (Section 2.3) was taken 10 min after ultrasonic treatment and imaged by TEM (Model EM10C-80KV, Germany). Elemental analysis of rubber samples was carried out by X-ray diffraction (XRD) analysis (Model Philips Expert-MPD, Netherlands). The size of ash nano-particles in the suspension (20 mg L^{-1}) was estimated using Zetasizer Instrument (Model Nano-ZS 90, Malvern).

2.3. Metal analysis

Two grams of ground tire rubber were extracted by a mixture (20 mL) of 4 M HNO_3, 1 M H_2SO_4, and 0.5 M DTPA. Also, 1 g tire rubber ash was dissolved in 20 mL 1 M H_2SO_4 and placed on an electric mixer at 120 rpm for 24 h. The concentrations of Fe, Pb, Cd, and Zn in the extract were measured using atomic absorption spectrophotometer (AAS) (Perkin-Elmer, Model 3030).

2.4. Nutrient solution culture

Seeds of cucumbers *Cucumis sativus* L. cv. Dominus were germinated in washed sand for two weeks. Composition of the nutrient solution was 0.88 mM K_2SO_4, 2.0 mM $Ca(NO_3)_2$, 0.25 mM KH_2PO_4, 1.0 mM $MgSO_4$, 0.1 mM KCl, 1 μM H_3BO_3, 0.5 μM $MnSO_4$, 0.2 μM $CuSO_4$, 0.02 μM $(NH_4)_6Mo_7O_{24}$, 100 μM FeEDTA and 2 mM MES [2-(N-morpholino) ethanesulfonicacid] adjusted to pH 6 with KOH as a pH buffer. All chemicals used in this experiment were analytical grade with a purity of $>98\%$. The pH of the nutrient solution was adjusted to 6.0. The beakers were wrapped with paper to keep out light. Each beaker was covered by a plastic sheet with two holes on the top. Roots of the seedlings were submerged in the nutrient solution. The nutrient solution was replaced twice a week. The daily photoperiod in the greenhouse was 12 h, with average daily maximum and minimum temperatures of 30 °C and 18 °C, respectively. The seedlings were precultured in the Zn-free nutrient solution for two weeks before adding treatments to allow healing of any root breaks when moving the plantlets from the sand to solution culture. Various concentrations of Zn (0, 1, 5, 25, 125 mg L^{-1}) were added into the nutrient solution in the form of $ZnSO_4 \cdot 2H_2O$ or tire rubber ash nano-particles (<50 nm size). Ten mg RANPs contained 1 mg Zn L^{-1}. RANPs were added into the nutrient solution, followed by water bath ultrasonic treatment (25 °C, 100 W, 40 kHz) for 1 h. The suspensions were stirred with a glass rod three times per day at 8 h intervals. The exposures to treatments lasted for 28 days and at the harvest time, roots and shoots were dried at 70 °C for 48 h, and weighed. Plant materials were digested by 2 M HCl and Zn, Cd, and Pb concentrations in the extracts were measured by AAS.

2.5. Microscopy observations

To observe the effect of RANPs on primary root tips of cucumber by light microscopy (Model E100, German), at harvest, the first 6 cm rootlets were longitudinally sliced (500 nm thick), thoroughly washed with deionized water and prefixed with alcohol (70%) and glycerin. Sliced root samples were washed in sodium hypochlorite (20%) and acetic acid and embedded in carmine for 15 min. The samples were then washed with deionized water and embedded in green methyl for 30 s.

Another sample (0–2 cm) of other root tips was thoroughly washed with deionized water, cut, dehydrated using freeze dryer (Model FD-4, Pishtaz Engineering Co., Iran), coated with gold for 60 s by a Sputter Coater (Model SCDOOS-Baltec, Switzerland), and then imaged by SEM (Model Phillips XL30 Robeahi, Netherlands).

Root samples were also prepared for TEM according to a standard procedure (Ni et al., 2005). The cucumber roots were prefixed in 2–4% glutaraldehyde, washed in 0.1 mol/L phosphate buffer (pH 7.0), profixed in 1% osmium tetrooxide, and dehydrated in acetone. Cross sections (65 nm thick) of the root tips were cut using a microtome (Model EM10C-80KV, Germany) with a diamond knife for TEM.

2.6. Statistical analysis

The nutrient solution culture experiment was set up in a completely randomized design with five treatments with three replicates. Treatments effects were analyzed by the analysis of variance using the GLM procedure. Means were compared using least significant differences (LSD) at $P<0.05$ (SAS Institute, 2000).

3. Results and discussion

3.1. Characterization of tire rubber nano-particles (RANPs)

The rubber ash contained about 10% Zn while concentrations of Cd and Pb in the rubber ash were below the detection limit of AAS. According to the USEPA (USEPA, 1999), the maximum acceptable ratio of Cd to Zn in zinc fertilizers is 1.4 mg Cd/kg Zn in the product. It has been reported that ground tire rubber and its ash has negligible amounts of Cd and Pb and can be used as pure and effective Zn-fertilizers in agricultural soils (Taheri et al., 2011). Zinc in the RANPs is mainly in the form of ZnO and ZnS-compounds that can easily release Zn for plant uptake. Rubber ash contained 1401 mg kg^{-1} Fe and negligible amounts of Si and Mn.