Ecotoxicology and Environmental Safety 165 (2018) 423–433

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Non-toxicity of nano alumina: A case on mung bean seedlings

Nisha Shabnam, Hyunook Kim*

Department of Environmental Engineering, University of Seoul, Seoul, Republic of Korea

ARTICLE INFO

Keywords:
Al_2O_3 nanoparticles
Al ions
Antioxidants
Oxidative stress
Vigna radiata

ABSTRACT

Wide use of Al_2O_3 nanoparticles (NPs) leading to their possible escape into environment and their interaction with living organisms demands immediate attention. We evaluated impact of nanoparticulate (Al_2O_3-NPs) and ionic (Al^{3+}) forms of aluminium on early seedling growth of *Vigna radiata*. While Al^{3+} inhibited growth of seedlings, Al_2O_3-NPs did not affect it negatively. Unlike enhancement in proline, malondialdehyde and H_2O_2 levels in roots and shoots induced by Al^{3+}, these stress markers remained unaltered by Al_2O_3-NPs. No signs of membrane damage were recorded in roots of seedlings raised in presence of Al_2O_3-NPs; this was witnessed from insignificant electrolyte leakage and Evans blue uptake. Activities of antioxidant enzymes, i.e., superoxide dismustase, catalase, guaiacol peroxidase in root and shoot were enhanced by Al^{3+}. However, they were unaffected by Al_2O_3-NPs. Al^{3+} enhanced levels of non-protein thiols, phenolics and ascorbate, with no alterations induced by Al_2O_3-NPs. These findings revealed that, Al_2O_3-NPs did not induce oxidative stress in seedlings. Seedlings raised in Al^{3+} showed higher uptake of Al than those grown in Al_2O_3-NPs; Al content was higher in roots. Al was not detected in shoots of seedlings grown in Al_2O_3-NPs. Lower translocation of Al in seedlings raised in Al_2O_3-NPs was due to adsorption/restriction of Al_2O_3-NPs on root surface. Al^{3+} caused ruptures on root epidermis of seedlings and inhibited root-hair formation, whereas no structural damage was caused by Al_2O_3-NPs. Our findings revealed that while ionic Al is highly toxic, nanoparticulate form of Al is non-toxic to growth of *V. radiata*.

1. Introduction

Distinct properties of materials at a nano level (< 100 nm), for examples, large surface area to volume ratio, small size and high reactivity, enable them to find applications in various industrial sectors and our daily lives (Handford et al., 2014). Owing to their wide applications, it is estimated that the global market for metal and metal oxide nanoparticles will surpass 10,000 t and 51,000 million USD by the end of 2026 in terms of production and values, respectively (https://www.futuremarketinsights.com/reports/metal-and-metal-oxide-nanoparticles-market).

Aluminium oxide or alumina (Al_2O_3) NPs are widely used in rocket propellants and explosives (Armstrong et al., 2003; De Luca et al., 2005), catalysis (Zacheis et al., 1999), electrosensors and electroanalysis (He et al., 2009), coatings (Landry et al., 2008), high performance ceramics (Tang et al., 2004), sunscreens (Lu et al., 2015), etc. Apart from their use as fillers and packing material (Sawyer et al., 2003), they find special applications in wastewater treatment technology through fabrication of smaller pore sized ultrafiltration membrane (Kim and Van der Bruggen, 2010; Savage and Diallo, 2005) for better performance index. Al_2O_3 NPs also serve as a solid phase extraction material for absorption, adsorption and preconcentration of heavy metal ions and other pollutants (Bhargavi et al., 2015; Ezoddin et al., 2010; Bhatnagar et al., 2010; Kumar et al., 2011). The extensive use of NPs raises a concern of their escape into the environment. Reports of silver NP release from fabrics during washing and the leaching of NPs from paints add to the apprehensions of NPs getting leaked into the environment (Benn and Westerhoff, 2008; Geranio et al., 2009; Kaegi et al., 2010; Zuin et al., 2014). Further, Gondikas et al. (2014) reported presence of Al_2O_3 NPs along with TiO_2 and ZnO NPs in the River Danube as a result of their release from sunscreens used by people during recreation activities.

Hence, it is essential to understand the effects that NPs can have on living organisms. Although there are numerous reports of NP toxicity

Abbreviations: CAT, catalase; DTT, dithiothreitol; EDTA, ethylene diamine tetra acetic acid; EDS, energy dispersive spectroscopy; GAE, gallic acid equivalent(s); GPX, guaiacol peroxidase; NP, nanoparticle; $NADP^+$, oxidized nicotinamide adenine dinucleotide (phosphate); NADPH, reduced nicotinamide adenine dinucleotide (phosphate); PVP, polyvinylpyrrolidone; SEM, scanning electron microscope; SOD, superoxide dismutase; TCA, trichloroacetic acid; Tris, tris-(hydroxymethyl)-aminomethane

* Corresponding author.
E-mail address: h_kim@uos.ac.kr (H. Kim).

https://doi.org/10.1016/j.ecoenv.2018.09.033
Received 15 June 2018; Received in revised form 2 September 2018; Accepted 6 September 2018
0147-6513/

on living systems, there has been limited research on the impact of Al_2O_3 NPs. Few *in vitro* studies carried out with mammalian cells showed cytotoxicity and genotoxicity of Al_2O_3 NPs on reproductive cells (Di Virgilio et al., 2010) and murine macrophages cell lines (Soto et al., 2005). Al_2O_3 NPs can potentially cross cell membrane (Radziun et al., 2011), alter immune repose (Braydich-Stolle et al., 2010) and pose a high risk of cardiovascular diseases through induction of pro inflammatory response in endothelial cells (Oesterling et al., 2008). Toxicity of Al_2O_3 NPs was also reported for a few micro-organisms and planktons (Hu et al., 2009; Strigul et al., 2009; Sadiq et al., 2011) with limited literature available on the effects of Al_2O_3 NPs on plants. The reports are restricted to seed germination and root elongation wherein both non-inhibitory/positive (Doshi et al., 2008; Juhel et al., 2011; Lee et al., 2010; Lin and Xing, 2007) and negative effects have been reported (Burklew et al., 2012; Yang and Watts, 2005; Yanık and Vardar, 2015; Yanık et al., 2017).

In this paper, we have evaluated phytotoxic effects of ionic and nano aluminium on *Vigna radiata* (mung bean), a popular leguminous crop consumed by a large population. Legumes are well known for their high protein content and their role in improving the fertility of soil. The findings presented in this paper reveal the non-toxicity of nanoparticulate Al to *V. radiata* compared to its ionic counterpart.

2. Materials and methods

2.1. Materials

Seeds of *Vigna radiata* (cv-SML-668) were procured from Indian Agricultural Research Institute, New Delhi, India. $AlCl_3$ and Al_2O_3 NPs were brought from Sigma-Aldrich (Saint Louis, MO, USA). Detailed characterisation of Al_2O_3 NPs is presented in Fig. 1. As per the transmission electron micrographs, nanoparticles were irregular shaped within size range of 20–60 nm (Fig. 1A,B). Energy dispersive X-ray spectrum showed peaks specific to Al thus confirming that nanoparticles were composed of Al (Fig. 1C). In XRD spectrum, the prominent peaks noted at 2θ angles were near 35.1 (104), 37.7 (110), 43.3 (113), 52.2 (024), 57.5 (116), 66.5 (214), 68.1 (300) which corresponded to α-Al_2O_3 (corundum), confirming that nanoparticles were crystalline (Fig. 1D). The other weak peaks noted in the XRD spectrum were that of θ-Al_2O_3. Selected area electron diffraction pattern also confirmed the crystalline structure of nanoparticles (Fig. 1E).

2.2. Methods

2.2.1. Raising seedlings of Vigna radiata in presence of nanoparticulate and Ionic aluminium

For nano particulate and ionic aluminium, Al_2O_3 NPs and $AlCl_3$ were used, respectively. Equivalent concentrations (25, 50, 75, and 100 mgL^{-1}) of nano particulate and ionic Al were prepared in distilled water on the basis of aluminium content in Al_2O_3-NPs and $AlCl_3$, respectively. Al content in these solutions were confirmed through inductively coupled plasma (ICP) emission spectrometer (details given below section). Al_2O_3 NPs were sonicated for 60 min for a uniform suspension to prepare a stock solution of 1000 mgL^{-1}. Whenever used, Al_2O_3 NPs stock solution was sonicated for 30 min.

V. radiata seeds were washed with detergent, rinsed with distilled water and surface sterilized for 3–5 min using 2N% sodium hypochlorite. The seeds were then rinsed with sterile distilled water under a laminar hood. 15 seeds were inoculated in autoclaved glass bottle containing glass beads and 20 mL of test solution of ionic/

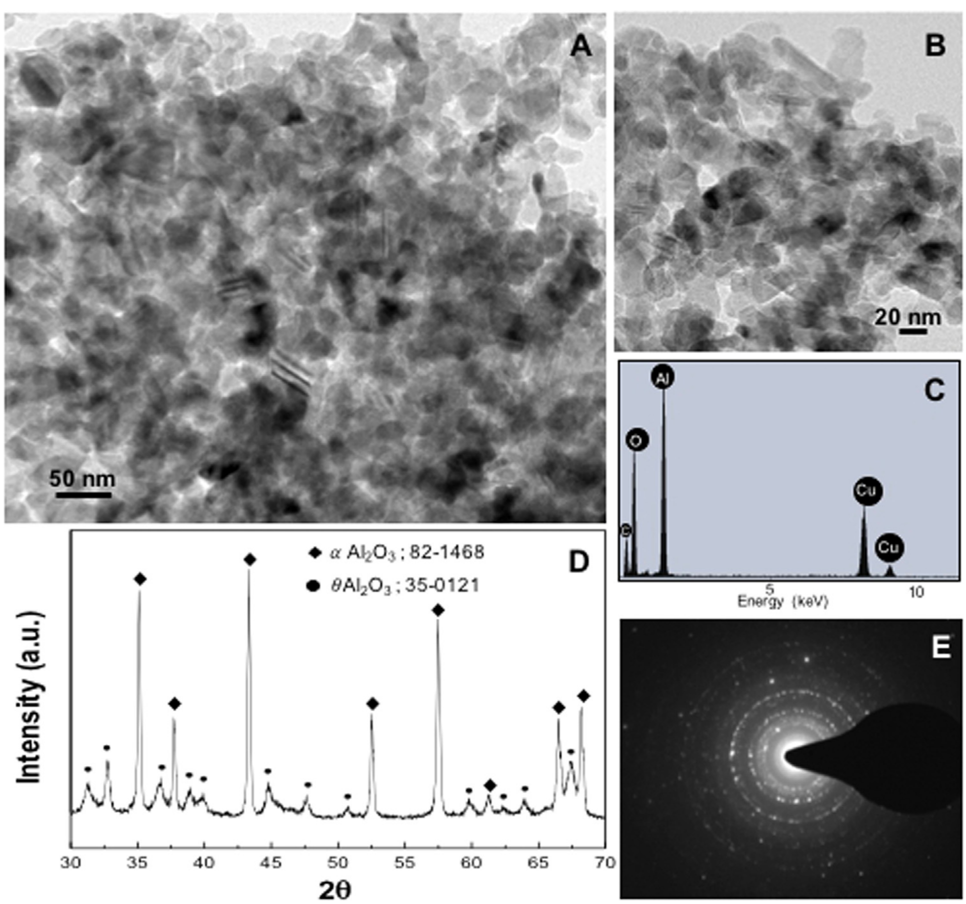

Fig. 1. Transmission electron micrographs (A, B), energy dispersive X-ray (C), X-ray diffraction (D), and selected area electron diffraction pattern (E) of Al_2O_3 nanoparticles.