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Toxicity evaluation of ZnO and TiO_2 nanomaterials in hydroponic red bean (*Vigna angularis*) plant: Physiology, biochemistry and kinetic transport

Shanaz Jahan[1,*], *Yatimah Binti Alias*[2,3], *Ahmad Farid Bin Abu Bakar*[1,*], *Ismail Bin Yusoff*[1]

1. *Department of Geology, Environmental and Earth Sciences, Faculty of Science, University of Malaya, Kuala Lumpur 50603, Malaysia*
2. *Department of Chemistry, Faculty of Science, University of Malaya, Kuala Lumpur 50603, Malaysia*
3. *University Malaya Centre for Ionic Liquids (UMCiL), University of Malaya, Kuala Lumpur 50603, Malaysia*

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ABSTRACT

The toxicity and kinetic uptake potential of zinc oxide (ZnO) and titanium dioxide (TiO_2) nanomaterials into the red bean (*Vigna angularis*) plant were investigated. The results obtained revealed that ZnO, due to its high dissolution and strong binding capacity, readily accumulated in the root tissues and significantly inhibited the physiological activity of the plant. However, TiO_2 had a positive effect on plant physiology, resulting in promoted growth. The results of biochemical experiments implied that ZnO, through the generation of oxidative stress, significantly reduced the chlorophyll content, carotenoids and activity of stress-controlling enzymes. On the contrary, no negative biochemical impact was observed in plants treated with TiO_2. For the kinetic uptake and transport study, we designed two exposure systems in which ZnO and TiO_2 were exposed to red bean seedlings individually or in a mixture approach. The results showed that in single metal oxide treatments, the uptake and transport increased with increasing exposure period from one week to three weeks. However, in the metal oxide co-exposure treatment, due to complexation and competition among the particles, the uptake and transport were remarkably decreased. This suggested that the kinetic transport pattern of the metal oxide mixtures varied compared to those of its individual constituents.

Introduction

Nanomaterials (NMs), due to their increased commercial applications, have entered aquatic and terrestrial ecosystems and produced toxicity toward living things, including plants. Previous phytotoxicological investigations revealed that the interactions of NMs with plants depend upon several factors such as NM composition, shape, surface area and surface charge (Carrola et al., 2016; George et al., 2009; Ma et al., 2010; Moussa et al., 2016; Nordmann et al., 2015; Soni et al., 2015; Zhao et al., 2015). Nonetheless, knowledge gaps about the effect of surface properties and the mode of NM exposure, such as single-oxide vs. co-exposed treatments, still exist in investigations on plant growth (Navarro et al., 2008). In comparison with other metal oxides, zinc oxide (ZnO) and titanium dioxide (TiO_2) NMs are widely utilized due to their superior electrical, catalytic and light absorption capabilities (Chen et al., 2017; Kumar et al., 2017). However, the comparative and competitive uptake, translocation and toxicity of ZnO and TiO_2 into plants have rarely been studied.

* *Corresponding authors. E-mail:* jshanaz01@gmail.com (Shanaz Jahan), a_farid@um.edu.my (Ahmad Farid Bin Abu Bakar).

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Previous phytotoxicological studies of NMs have focused on edible plants (Ebbs et al., 2016; López-Moreno et al., 2010; Mukherjee et al., 2014; Nordmann et al., 2015; Schwabe et al., 2015; Zuverza-Mena et al., 2015). However, the conclusions obtained from these studies are contradictory, and sometimes even opposing interpretations have been drawn (Aslani et al., 2014). For example, a research group (Servin et al., 2013) reported that TiO_2 had a strong impact on cucumber plants and increased the chlorophyll content and catalase activity in cucumber leaves; whereas another study indicated that TiO_2 had very minimal and relatively weak impacts on plant growth compared to ZnO nanoparticles (Landa et al., 2012). Similarly, another study (Song et al., 2013) revealed that TiO_2 showed no evidence of phytotoxicity in tomato plants even at 5000 mg/kg. Furthermore, no dose-dependent changes in seed germination and seedling growth were observed in wheat seeds exposed to TiO_2 nanoparticles (Feizi et al., 2012).

Additionally, in all the above-mentioned reports, even though the toxic effects of individual metal oxides may be well established, the environmental risks posed by metal oxide mixtures are difficult to predict at this time. Nonetheless, the results obtained from a limited number of pioneering studies suggest that the toxic potential of a metal oxide mixture is distinct from those of its individual constituents (Tong et al., 2015). Another important aspect that is poorly understood is the evaluation of uptake kinetics. Kinetic data will help provide knowledge on how NMs are taken up by plants, including the short-term and mid-term uptake capacity and the speed of NM transport into the plants. Therefore, evaluation of this phytotoxicological parameter is crucial and needs to be explored (Ma et al., 2010).

Hence, the objectives of this study are: (1) to determine the comparative physiological and biochemical impacts of ZnO and TiO_2 on red-bean plant growth; (2) to investigate the effect of the exposure period on the kinetic uptake and translocation of both metal oxides; and (3) to evaluate the competitive uptake and translocation of ZnO and TiO_2 in metal oxide mixtures. In this study, we selected the Red bean as a model plant to evaluate the toxicity of ZnO and TiO_2 since it widely consumed in a variety of recipes among most of the Asian countries, although none of the previous research has focused on elaborating the effect of metal oxides on plant growth. To determine the toxicity of ZnO and $TiO_{2,}$ the concentration range of 0–200 μg/mL was used. These wide ranges of metal oxide concentrations were examined to determine the effects of a sub-lethal dose on plant physiological and biochemical characteristics. We employed hydroponic cultivation because of its faster growth rate, lower water consumption, lack of a fertilizer requirement and lower space demand for plant growth (AlShrouf, 2017). To the best of our knowledge, this is the first report describing comparative and competitive responses of the red bean model plant to metal oxides in single and co-exposed treatments.

1. Materials and methods

1.1. Synthesis of metal oxides

ZnO and TiO_2 were synthesized following previously published reports (Brauer and Szulczewski, 2014; Pimentel et al., 2014) with slight modifications. The quality, purity and stability of synthesized materials were ensured by taking quality assurance and quality control steps during the overall synthetic procedures. Briefly, in the synthesis of ZnO, 3.9 g of Zinc acetate dihydrate solution (0.45 mol/L) prepared in deionized water was mixed with NaOH solution (8 mol/L) with constant stirring, which yielded a transparent solution of zincate ions, $Zn(OH)_4^{2-}$. The mixture was allowed to cool down to room temperature, then 5 g of polyethylene glycol-PEG (6.9 mmol/L) in 90 mL of deionized water was mixed with 12 mL of $Zn(OH)_4^{2-}$ solution. The resulting mixture was heated hydrothermally at 100°C for 5 hr. The white ZnO precipitate formed was washed several times with 2-propanol followed by deionized water by centrifugation at 4000 r/min for 10 min.

For the synthesis of TiO_2, 15 mL of the titanium isopropoxide solution was mixed with 10 mL of 2-propanol (solution A). In a beaker, 100 mL of chilled deionized water was added, and the pH was adjusted to *ca.* 3 with HCl (solution B). Dropwise addition of solution A to solution B with vigorous stirring at 1100 r/min yielded a white precipitate of TiO_2. The precipitate was washed thrice with deionized water and centrifuged at 3000 r/min for 15 min to remove unreacted precursors. The metal oxides were oven-dried at 60°C and characterized by field emission scanning electron microscopy, FESEM (SU8220, Hitachi, Japan) and their size distribution curves were calculated. Homogenized suspensions of both metal oxides (0–200 μg/mL) in deionized water were prepared by ultrasonication (LUC-405, Powersonic, Korea) in a water bath at 25°C for 1 hr. The concentration of dissolved Zn(II) and Ti(IV) ions in the suspensions were immediately determined by ICP-MS analysis. The average diameters of metal oxide particles in the suspensions were determined by FESEM analysis, and the surface potential was investigated using a Zetasizer (zn3600, Malvern Zetasizer, UK) in low-volume disposable cuvettes, and the mean value of three measurements was recorded. To investigate the aggregation or sedimentation in each suspension, the stability of ZnO and TiO_2 in suspensions was determined by a UV–Visible spectrophotometer (UV-3600, SHIMADZU, Japan) with a 1 cm path-length cuvette. In particular, the changes observed in the optical absorbance at 376 nm for ZnO and 375 nm for TiO_2 were obtained as a function of solution pH. The details of the absorption maxima of both metal oxides are discussed in our previous report (Jahan et al., 2017).

1.2. Seed germination

Red bean seeds were soaked in formaldehyde 3% (V/V) for 15 min to remove fungal contaminants (Peralta-Videa et al., 2002) and washed thrice with deionized water. For the germination test, around 12 seeds were placed on filter paper in a borosilicate glass Petri dish (150 mm × 30 mm) and 10 mL of metal oxide suspension was added (separate germination tests were carried out for each set of metal oxide concentrations). The seeds were covered with another filter paper, wrapped with aluminum foil and kept in an incubator at 30°C for 5 days. All samples were replicated three times and mean values ± SE (n = 3) were recorded. Seeds were considered germinated when 65% of root controls were at least 5 mm long (Fig. 1, step 1), according to US Environmental Protection Agency guidelines (USEPA, 2012). Germination data were collected and percent

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