



Effects of Nano-TiO₂ particles on bioaccumulation of ¹³³Cs from the contaminated soil by Soybean (*Glycine max*)



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ARTICLE INFO

Article history:

Received 10 December 2016

Received in revised form 27 October 2017

Accepted 22 February 2018

Keywords:

¹³³Cs

Nano-TiO₂

Accumulation

Soybean

XPS

FTIR

ABSTRACT

This study investigated the effects of nano-TiO₂ particles on cesium uptake by soybean plant, which was conducted in a plant growth chamber with adding different concentrations of ¹³³Cs to the soil. This study identified compositional and functional group changes of the elements contained in the soybean root and shoot due to the accumulation of ¹³³Cs. With use of nano-TiO₂, the accumulation of ¹³³Cs identified in the shoot (731.7 μg/g dw) was higher than that in the root (597.8 μg/g dw). The Cs 3d peaks identified in the XPS spectrum analysis of the shoot and root biomass could be an evident for ¹³³Cs accumulation. The appearance of the new FTIR peaks on the root and shoot biomass can be explained by new bond formation of ¹³³Cs or nano-TiO₂ with biomass matrix. Therefore, the application of nano-TiO₂ in Cs contaminated soil can significantly enhance ¹³³Cs uptake and its accumulation in plants.

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1. Introduction

Radioactive nuclides (¹³⁴Cs and ¹³⁷Cs) have fallen over eastern Japan during the accident of Fukushima Daiichi atomic power plant. This incident happened by the breakdown of cooling system tempted by Tohoku earthquake and tsunami that had occurred on March 11, 2011 (Yoshida and Takahashi, 2012). Approximately, 22.5 million tons of wastes have been collected in year 2012 from the three major disaster-suffering areas of Iwate, Miyagi and Fukushima Prefectures; however, the huge amount of radioactive nuclides have also deposited in natural soil and plant system (Yasunari et al., 2011). These radioisotopes have been attracted important consideration in environmental science and engineering due to their relatively high fission yield, long half-life, and high mobility in organisms (Yan et al., 2013). Cesium radioisotopes can be transferred into crops during direct deposition in soil through the plant roots system. Therefore, the accumulation of radioisotopes in vegetation and soil is the preliminary step of radioisotope transport in the terrestrial environment and in food chains (Kinnarsley et al., 1997). Various studies have been carried out on cesium translocation from plant roots to other part of plant (Moogouei et al., 2011; Kang et al., 2012; Yan et al., 2013).

Applications of nanoparticles (NPs) with size less than 100 nm have significantly increased in current years (Du et al., 2011). Nano-TiO₂ is the most widely used metal oxide particle in the world and thus its global production increased up to 10,000 tons per year (Servin et al., 2013). It has been considered that nano-TiO₂ particles are currently produced high demand for their multiple applications. Nanoparticle mobility in soils depends on its size, shape, agglomeration state and chemical composition and soil composition can also affect mobility of particles (Nowack and Bucheli, 2007). Rutile nano-TiO₂ (2.5%) stimulates the germination of spinach seeds, while 0.25% rutile nano-TiO₂ increases the spinach photosynthesis by enhancing cyclic and linear photophosphorylation as a results of increasing to Mg²⁺-ATPase activity (Gao et al., 2013). Rutile nano-TiO₂ promotes the spinach growth by defensive the chloroplast membrane structure from reactive oxygen species, consequently, improving the activity of antioxidant enzymes (superoxide dismutase, catalase and peroxidase) (Gao et al., 2013). According to the Yang et al. (2006), nano-TiO₂ could improve the activity of nitrate reductase to hasten the change of inorganic form of nitrogen (NO₃-N and NH₄⁺-N) to organic form of nitrogen. Soybean plants [*Glycine max* (L.) Merr.] are chosen for the experiment because soybean is one of the most vital and most commonly harvested edible crops worldwide (Yan et al., 2013). Soybean is an essential food in diets of many parts of the world. Soybean and other beans that powerfully accumulate heavy metals and radionuclides (Shinonaga et al., 1999; Zhuang et al., 2013).

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Fig. 1. Pictorial view of plant growing in the plant growth chamber.

However, the data on the effect of nano-Titanium dioxide (nano-TiO₂) particles on the bioaccumulation of ¹³³Cs by Soybean from the contaminated soil is rather limited. To address the urgent issues regarding Cs-contaminated land in Fukushima Prefecture, the present study was conducted to examine the potential of remediation of Cs (stable ¹³³Cs) from the contaminated soil using Soybean plants in the presence of TiO₂ NPs. We examined the pattern of translocation and accumulation of Cs in the parts of this plant.

2. Materials and methods

2.1. Chemicals and reagents

Cesium chloride, NH₄NO₃ and KH₂PO₄ were purchased from Daejung Chemical and Metals Co. LTD., South Korea. Nano-TiO₂ particles (mixture of rutile and anatase, size < 100 nm) were purchased from Sigma-Aldrich, USA.

2.2. Plant growth chamber (PGC) design

The PGC was designed with wooden frame (length=2 m, width=1.0 m, and height=1.6 m, entire area=2.0 square meter) covered by polypropylene plastic (two fold). Inside the PGC, two benches (2.0 m length and 0.2 m width) were placed. For proper aeration, 10 holes (10 mm) were created in upper part of the PGC, and two windows (30 × 25 cm) were made for cross ventilation. Inside the PGC, humidify was kept about 50–80% through employing four trays beneath the benches which were filled with pebbles and water. There were two incandescent bulbs of 500 W and one mixed light source of dissimilar wavelength are installed inside the PGC for the photosynthesis of plants (Geo Lighting, Global light Company, South Korea). The PGC was placed near the window for proper aeration. In the morning, plants were open by sunlight coming through window. The maximum temperature was checked 28 ± 2 °C during the day time and minimum temperature 20 ± 2 °C observed during the night time in side PGC. Plants were exposed in light with natural illumination (period of cycle 11 14 h of light and 10–13 h of darkness). Fig. 1 shows the inside pictorial view of the PGC.

2.3. Cultivation of soybean

In July, 2014, the experimental soil was collected at 0–20 cm depth from the agricultural field near the University of Ulsan, South Korea. The soil was air dried and then followed by removal of twigs and stones manually as well as sieved through a 2 mm sieve. The

physicochemical characteristics of the soil were then analyzed. In this study, Soybean plants were cultivated in plastic pots (240 mm inner diameter and 200 mm depth) filled with 3.0 kg of dry soil. The chemical fertilizers were added at rates of 200 mg/kg of N, 150 mg/kg of P, and 200 mg/kg of K as analytical grade NH₄NO₃ and KH₂PO₄ reagents to each pot and then fertilizers were mixed properly before seedling. The water was added to reach the maximum water holding capacity of the soil; watered pots were allowed to equilibrate for two week (Yan et al., 2013). Seeds were shown in pots from 3 to 5 cm depth. Eight seeds were sown in each pot on July 31, 2014 and thinned for 3–4 plants on the fourth day after germination. All pots were watered regularly every third days with constant amounts. Plants were grown in a PGC throughout the experiment, and watered with deionized water. The PGC did not have climate control but operated at an ambient temperature.

Cesium contamination (100–300 mg/kg) of the soil was done by adding cesium chloride salt along with addition of chemical fertilizers before sowing Soybean. Nano-TiO₂ particles were added in the range of 100–300 mg/kg to the soil. Seeds of Soybean (*Glycine max*) were washed in tap water. The details of experimental treatments are provided in Table 1.

2.4. Preparation of Nano-TiO₂ particles suspensions

The amount of TiO₂ necessary to prepare 100, 200 and 300 mg TiO₂/kg of soil was suspended in 400 mL of Milli-Q deionized water by sonication in a water bath for 30 min (WiseClean, DAIHAN Scientific, Seoul, South Korea) at 30 °C with occasional stirring.

2.5. Morphological analysis

The whole plant was used for biomass measurement after sowing 30 and 60 days. The plant samples was washed with distilled water to remove the adhering soil particles and separated from the above-ground parts and the root parts with scissors. Firstly, samples were air-dried in cool and well ventilated places, then were oven dried (68 °C ± 2 °C, 24 h). The dry weight of each part was weighed to calculate the biomass.

2.6. Biochemical analysis

Fresh leaves were harvested from the selected plants treated with various amendments for estimation of leaf relative water content (RWC), chlorophyll content, protein, proline and malondialdehyde (MDA) at 15th, 30th and 60th day of experimentation.

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