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Uptake, transportation, and accumulation of C_{60} fullerene and heavy metal ions (Cd, Cu, and Pb) in rice plants grown in an agricultural soil☆

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ABSTRACT

The mutual influences of C_{60} fullerene (C_{60}) and heavy metal ions (Cd, Cu, and Pb) on the uptake, transportation, and accumulation of these coexisting pollutants in four rice cultivars planted in agricultural soil were investigated during the whole life cycle of rice. The biomass of the rice plants was not affected significantly by the presence of C_{60}. C_{60} exposure exerted different impacts on the bioaccumulation of Cd, Cu, and Pb in various rice tissues. For example, the bioaccumulation of Cd in rice 9311 panicles was significantly decreased ($p < .05$) when it was exposed to 1000 mg/kg C_{60}, whereas the changes of Cu and Pb levels in panicles were not statistically significant. C_{60} was absorbed by rice roots and transported to the stems and panicles, and it tended to form aggregates in rice tissues. C_{60} concentrations in the roots, stems, and panicles of the four rice cultivars that were harvested after a 130-day exposure to 600 mg/kg C_{60} were 40–292, 4.4–24.5 and 0.077–1.2 mg/kg (dry weight), respectively. C_{60} and heavy metal ions exhibited different uptake and transportation mechanisms, which depended on the rice cultivar, soil heavy metal ion concentration, and C_{60} exposure time and concentration. For example, the average C_{60} in the four rice cultivars was increased sharply, from 47.4 to 196.3 mg/kg from the tillering to booting stages, whereas Cd levels increased only slightly, from 23.1 to 25.9 mg/kg. The study demonstrated that the bioaccumulation of C_{60} and heavy metal ions under co-contamination scenario differs from under single contaminant. The accumulation of C_{60} in rice panicles may increase the concern of food safety.

1. Introduction

The application of various engineered nanoparticles (ENPs) in modern agriculture, food technology, and soil remediation has increased the potential for ENPs to enter agricultural soil, where they have various effects on terrestrial plants (Deng et al., 2014; Du et al., 2017; Zahra et al., 2015). ENPs enter crops mainly through root uptake and are then transported into other tissues. The presence of ENPs could lead to morphological, physiological, genetic, and epigenetic changes in agricultural crops over time, and such changes may subsequently affect crop growth, yield, or nutritional status (Rizwan et al., 2017; Servin et al., 2015; Tolaymat et al., 2017). Properties, including surface charge, particle size, and the bioavailability of ENPs, could change over time after their introduction to soil (Hotze et al., 2010). Notably, changes in these properties could subsequently influence interactions between ENPs and plants. It has been reported that particle size and surface coating strongly influence the uptake of Au nanoparticles by tobacco (*Nicotiana tabacum* L. cv *Xanthi*) (Judy et al., 2012b). The fundamental effects of ENPs on agroecosystems cannot be precisely described unless investigations are conducted in agricultural soils, particularly under field conditions. The interactions between ENPs and crops in agricultural soils are unclear, and comprehensive studies are still urgently needed.

Fullerenes are carbon based nanoparticles and have been frequently detected in soil samples at levels ranging from ng/kg to low μg/kg (Carboni et al., 2016; Sanchís et al., 2013, 2015). The adsorption of C_{60} on soil is highly dependent on soil organic matter content and the transport of C_{60} aggregates in natural soils is limited (Li et al., 2008; Wang et al., 2010), with C_{60} not very bioavailable to soil microbes and therefore likely to persist in soil

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(Avanasi et al., 2014). It has also been reported that fullerene and its derivatives have different effects on the uptake, transportation and accumulation of coexisting pollutants in agricultural crops; such effects are in addition to those related to its own uptake by such crops (De La Torre-Roche et al., 2012; Husen and Siddiqi, 2014; Lin et al., 2009). For example, the presence of fullerene promoted the uptake of trichloroethylene by cottonwood (*Populus deltoides*) (Ma and Wang, 2010); the uptake of *p,p*′-DDE by zucchini (*Cucurbita pepo* L.), soybean (*Glycine max* L.) and tomato (*Solanum lycopersicum* L); and the accumulation of chlordane in tomato and soybean to different extents (De La Torre-Roche et al., 2012; De La Torre-Roche et al., 2013) Such effects ranged from 30% to 65%. Conversely, C_{60} had little impact on the uptake and transportation of weathered *p,p*′-DDE in zucchini (Kelsey and White, 2013), and the presence of C_{60} completely suppressed the uptake of DDT and its metabolites by corn (*Zea mays*) and tomato (De La Torre-Roche et al., 2013). These results indicate that the effects of C_{60} on the bioaccumulation of coexisting pollutants by crops vary substantially among different pollutants. However, most of these studies considered only a short exposure time (from several days to a month), and therefore our understanding of the bioaccumulation of ENPs and their effects on the uptake of coexisting pollutants by terrestrial grain crops particularly during their whole life cycle (from seedling to full maturity), is still quite limited.

Rice is one of the most important food crops, particularly in Asian countries, and contamination of rice by heavy metals or metalloid ions, including Cd, Cr, Pb, Cu, and As, has been frequently reported (Guo et al., 2017). To date, only three studies have reported the interactions of C_{70} (Lin et al., 2009), CeO_2 (Rico et al., 2013) and CuO (Peng et al., 2017) with rice plants in agricultural soils during the whole life cycle of rice. All three studies focused on the accumulation and transformation of corresponding ENPs in rice tissues and their effects on the quality of rice grains. The uptake and distribution of heavy metal ions and ENPs by rice plants under a co-contamination scenario have yet to be clarified and may be very different from those under conditions where a single contamination is present.

In this study, C_{60} fullerene was chosen as an ENPs proxy because of its widespread application and because it could be quantified by chromatographic analysis. The uptake, transportation, and bioaccumulation of C_{60} fullerenes and selected heavy metal ions (Cd, Pb and Cu) in four rice cultivars were investigated in an agricultural soil for approximately 4 months. The first objective was to quantify C_{60} and heavy metal ion concentrations in rice tissues, particularly in its panicles, under a co-contamination scenario. The second objective was to assess the mutual influence between C_{60} and heavy metal ions. The study revealed the effects of C_{60} on the uptake of heavy metal ions in soils under practical environmental conditions and increases our understanding of ENPs–grain crop interactions in the terrestrial environment.

2. Experiments and methods

2.1. Chemicals, soil, and rice plants

2.1.1. Chemicals

C_{60} fullerene (99%) was purchased from Suzhou Dade Carbon Nanotechnology Co., Ltd (Suzhou, China). Cadmium chloride (analytically pure), nitric acid (guaranteed reagent) and hydrogen peroxide (guaranteed reagent) were purchased from Sinopharm Chemical Reagents (Shanghai, China). All organic solvents (HPLC grade) and other chemicals were of the highest purity commercially available.

2.1.2. Soils and rice plants

The agricultural soil (water content 22%) used in the experiment was collected from Qianjiang (Hubei, China) at a depth of 20 cm. The Cd, Cu, and Pb background concentrations in the soil were 0.3, 24, and 20 mg/kg (dry weight), respectively. Other soil properties are presented in Table S1 (Supporting Information, SI). Large rocks, bricks, and plant residues were removed from soils, and no further treatment was carried out before its use in exposure experiments.

Exposure experiments were conducted at a native Cd concentration (0.3 mg/kg) and a high spiked Cd concentration (3.2 mg/kg). Specifically, a 10-kg soil sample was weighed and added into a 15-L bucket. Then, 2 L tap water was added to the buckets, and the soil was stirred for 15 min. Next, 1 L deionized water or 1 L $CdCl_2$ solution (Cd^{2+} is 30 mg/L) was added to the bucket, and the dispersion was stirred for another 15 min. These soils were referred to as native soil and Cd amended soil in latter application. A C_{60} suspension was slowly added to the buckets, and the resulting soil slurry was mechanically stirred (approximately 50 rpm) to achieve a homogeneous mixture. The final soil doses of C_{60} were 200, 600, and 1000 mg/kg. The buckets with spiked soil were then placed in a non-parameter controlled (i.e., under natural light and temperature) greenhouse with a waterproof roof for 2 weeks to stabilize the soil. Although the concentration of C_{60} fullerene applied in the present work was much higher than its detected concentration in soils, it enabled comparisons with previous studies where similar C_{60} or other ENP concentrations had been applied (Du et al., 2017; Lin et al., 2009; Peng et al., 2017; Rico et al., 2013).

Four rice cultivars *Oryza satia. L. indica* (9311, Luoyou 8 (LY8), Yuetai B (YB)), and *Oryza satia. L.Japonica* (Nipponbare (Nip)), were used in the present work. Seeds of the four rice cultivars were obtained from the College of Life Sciences, Wuhan University. Rice seeds were germinated under moist conditions at room temperature for 48 h. Then, the germinating seeds were symmetrically spread on a control soil and covered up with a piece of plastic membrane until 4–5 pieces of leaves emerged (approximately 3 weeks). Similar seedlings were then selected and used for exposure experiments.

2.2. Rice crops exposure assay

In mid-April 2015, the seedlings of four rice cultivars (9311, LY8, YB, and Nip) were transplanted into buckets containing the aforementioned treated soils (three replicates per treatment). Rice was planted in Cd amended soils (Cd: 3.3 mg/kg) with C_{60} (200–1000 mg/kg), native soils (Cd: 0.3 mg/kg) with 600 mg/kg C_{60}, or native soils without C_{60}. The details of the experimental assay are provided in Table S2 (SI). During cultivation, the water content in each bucket was adjusted to a uniform content (approximately 6 cm above the soil surface) by regular watering until the maturation stage. The cultivation experiment is depicted in Fig. S1 (SI). Rice samples were harvested from buckets in the tillering stage (31 days after transplanting), booting stage (75 days after transplanting), heading stage (100 days after transplanting), and full ripe stage (130 days after transplanting). The samples were then washed carefully with deionized water and dried at 50 °C for 30 h; then, the roots, stems and panicles from three independent rice plants were recovered and mixed separately. The dried root, stem or panicle samples were cut into small pieces and well mixed through mechanical disintegration. These samples were stored at 4 °C until use in the analysis of heavy metal ions and C_{60} fullerene concentrations.

2.3. Sample pretreatment

The extraction of C_{60} from rice samples was conducted as follows. Root, stem or panicle samples (0.2–3 g of dry biomass) were placed in a 60-mL PTFE vessel, and 15 mL toluene was then added.