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Excessive uptake of heavy metals by greenhouse vegetables

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ABSTRACT

The accumulation of some harmful elements in greenhouse vegetables has caused a serious threat to human health. Comparing tests with cucumber (*Cucumis sativus* L.) and tomato (*Lycopersicon esculentum*) were conducted between greenhouse (GH) and open field (OF) cultivation with objective to determining the effects of greenhouse cultivation on transport and distribution of Cu, Zn, Fe, Mn, Pb, and Cd in soil–vegetable system. The results showed that Cd, Mn and Zn contents in most tissues of the vegetables in GH were significantly higher than those in OF, Fe contents reduction occurred in all parts of GH plants, while Pb contents decreased only in aboveground parts of GH plants. Most elements bioaccumulated in GH vegetable roots, except Fe, but exhibited lower transportation from root to aboveground parts in GH than in OF. Fe fractions extracted with ethanol and acid expressed significantly higher percentages in some parts of GH vegetables than in OF vegetables. Element interactions between Fe, Cd and Mn were significant and played an important role on metal elements transportation in vegetables. In conclusion, greenhouse cultivation could threaten vegetable production safety through changing the transportation of most metals in soil–vegetable system.

1. Introduction

Heavy metals, being non-biodegradable and persistent in the environment, can reach toxic levels in vegetables, thus decreasing both yield and quality (Sharma et al., 2009). The problem of increasing contribution of daily intake of heavy metals from vegetables has to be considered (Hu et al., 2014). Usually, we attributed heavy metals pollution of vegetable to the environmental pollution, such as wastewater irrigation, excessive agricultural chemicals applying, or atmospheric pollutants deposition. When greenhouse production system (GH) is gradually becoming the mainstream of the facility agriculture, researchers found it was also a matter of course that not only elevated levels of heavy metals in soils (Cai et al., 2015; Fytianos et al., 2001), but also excessive heavy metals contents in vegetables (Collings et al., 2010; Li et al., 2009).

However, facility agriculture as greenhouse created a comfortable but different growing environment for plants compared with open fields as providing higher temperature (Park et al., 2012) and moisture levels but lower light intensity (Gao et al., 2012). Environmental scientists paid more attention on the levels and sources of trace element in the agricultural system (Ramos-Miras et al., 2011). They revealed that greenhouse production practices increased accumulation of the trace metals, particularly Cd, Zn, and Cu in soils and the accumulation of Cd and Zn was mainly from organic greenhouses, which associated with application of manure (Yang et al., 2013). Previous study showed high Cd accumulation in leaf vegetables due to the lowered soil pH and enhanced metal availability in greenhouse vegetable production (Yang et al., 2014), and the increased Cd transfer from root to the aboveground parts causing from film mulching (Li et al., 2012).

The distribution of heavy metals in plants is selective. Some toxic or excessive heavy metals were more like distributed in less important tissues, such as cell wall, vacuole, and leaf apoplast (Blinda et al., 1997; Küpper et al., 1999). Heavy metals bonding to various inorganic or organic anions have different solubility and biological activity (Cobbett, 2000). For instance, a Cd-sensitive barley genotype had a larger amount of Cd in inorganic and water-soluble forms than the Cd-resistant genotypes (Wu et al., 2005). A common sequent extraction method was to divide heavy metals into six fractions using extracting agents with gradually increasing extraction capabilities, in turn, were 80% ethanol, deionized water, 1 mol L^{-1} NaCl, 2% acetic acid, 0.6 mol L^{-1} HCl, and HNO_3–$HClO_4$ (3:1, v/v) (Wang et al., 2008; Zeng et al., 2010).

Nevertheless, little information is available on what caused the differences of transportation of metals between greenhouse and tradition agricultural vegetable plants. Thus, deeply understanding the distribution, translocation and accumulation of heavy metals in soil-vegetable system under the facility cultivation is important for assessment of vegetable security induced by heavy metals and its safety production control. The aims of this study were to determine the effects of greenhouse on soil properties, the translocation and distributions of

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Cu, Zn, Fe, Mn, Pb, and Cd in soil-cucumber and soil-tomato systems, and to reveal if and how greenhouse cultivation affects the transport of metals.

2. Materials and methods

2.1. Experimental sites and design

Field experiments were designed and conducted in 2010 at Hu-houtou Village (29° 44′ to 30° 12′ north latitude, 119° 25′ to 120° 09′ east longitude) of Fuyang in Hangzhou, Zhejiang Province, China. The experiment site was in rural areas and about southwest 18 km shortest distance to the beltway of Hangzhou city. The local soil was fluvo-aquic soil developed on marine sediments. In order to eliminate other effects resulting from long-term greenhouse (GH) cultivation, such as accumulation of trace metals in soil (Yang et al., 2013), a field, which had never been used for greenhouse cultivation and had little heavy metal pollution, was requisitioned in our experiment.

In the field, two greenhouses were constructed with 40 m long, 8 m wide and 2.5 m high, covered with 0.1 mm thick and colorless polyethylene, one for cucumber and the other for tomato. The size of the greenhouse was the most common one in China, in order to have the common heat preservation effect. Hundreds of cucumber (*Cucumis sativus* L.) and tomato (*Lycopersicon esculentum*) seedlings with seven to eight leaves were both divided into two groups for transplantation. For both tomato and cucumber seedlings, a group were grown in black polyethylene mulched soil in greenhouse, marked as $GH_{cucumber}$ and GH_{tomato}, respectively. There were four one-meter-wide ridges in each greenhouse with 0.6 m space between ridges, two columns of seedlings in each ridge, and the entire seedling plots were spaced regularly, about 30 cm apart. The other cucumber group about 50 seedlings was grown in two columns in the open fields, which were beside the greenhouse as control, marked as $OF_{cucumber}$, and so did the other tomato group and marked as OF_{tomato}. Passive ventilation in GH was dependent on sunlight as a primary energy source or wind when half opened. Irrigation water was from a brook nearby. Kept all other growing conditions of the two cultivations the same during the whole growth periods.

Compared with the OF, the GH obviously characterized with higher temperature and lower illumination according to our records of a week during the experiments. The temperature, measured with a mercury thermometer, was averagely 2 °C higher inside the GH than that of the OF at the same time, while the illumination intensity measured with a digital illuminance meter (1336A, TES, Taiwan, China) was usually two times lower than that outside. In addition, the average concentration of CO_2 was 636 ppm and average percentage of O_2 was 31.2%, respectively, which were higher than their averages in the atmosphere. The concentrations of CO_2 and O_2 were measured with a carbon dioxide sensor (TEL7001, Telaire, USA) and a portable oxygen measuring instrument (CY-12C, Longtuo, Shanghai, China), respectively. The detail temperature, illuminance and air information were documented in the supplement file.

2.2. Sampling and pretreatments

For each vegetable, the sampling points scattered uniformly along the double diagonal lines in ten replicates for GH, and along a single diagonal line in three replicates for OF. The whole cucumber and tomato plants and the corresponding rhizosphere soils were sampled 3–5 months after transplanted with some fruits ripened. The soil closely adhering to the root were removed by shaking the root gently and collected as rhizosphere soil. Air-dried the soil samples at room temperatures, and then ground to pass through 100-mesh plastic sieves and homogenized. The plant samples were thoroughly washed with tap water and subsequently with deionized water to remove adhered soil and plant debris, and then were blotted with tissue papers. Subsampled each cucumber or tomato plant into roots, stems, leaves and fruits. Freeze-dried cucumber and tomato fruits due to their high water content, while other subsamples, including roots, stems and leaves, were oven-dried separately at 70 °C to constant weights. The dried plant subsamples were ground and passed through 100-mesh plastic sieves individually. Stored the pretreated plant and soil samples in sealed plastic bag at room temperature prior to analysis.

2.3. Sample analysis

Soil moisture content was determined based on weight loss of 10 g fresh soil oven-dried at 105 °C. Soil pH was determined using a glass electrode (PHS-3C, Leici, Shanghai, China) in aqueous suspensions of 1:2.5 (soil: water, w/v). Soil organic matter (SOM) content was determined by the potassium dichromate-dilution heat titration method (Chen et al., 2000). Cation exchange capacity (CEC) was determined by the $BaCl_2$-H_2SO_4 method (ISO 11260–1997).

Chlorophyll content of leaf was determined by spectrophotometric method (Harris, 1989). The stomatal density in leaf was counted under an optical microscope (YS100, Nikon, Tokyo, Japan).

The total contents of iron, manganese and other metals in soils and plant tissues were determined by flame atomic absorption spectroscopy or with graphite furnace where necessary (AA800, Perkin Elmer, USA) after microwave digestion (MDS-8, SINEO, Shanghai, China). Samples (0.1 g of soil or 0.2 g of plant tissue) were digested with the mixture of 10 mL of HNO_3, 2 mL of H_2O_2 and 1–2 mL of HF under different programed temperature of the microwave. The soil samples were successively digested under 120, 150, 180, 210 °C for 8, 5, 5 and 45 min, respectively, while the plant samples were under 130, 170, 180 °C for 8, 6 and 30 min, respectively. Then uncovered the Teflon vessels, and evaporated the contents until they were almost dry. Repeat the evaporation with 2 mL of HNO_3. HF was used in plant sample digestion in order to remove the silica in cortical cell and cell wall, especially the root and the stem samples. Detection limits were defined as three times the standard deviation of ten runs of blank measurements, and they were 10, 10, 1.0, 0.6, 0.6, 0.1 μg L^{-1} for Cu, Zn, Fe, Mn, Pb and Cd, respectively.

Metal elements associated with different fractions in plant were sequentially extracted using a following simplified three steps method, which was modified from the previous method (Wang et al., 2008), by merging the intermediate steps as one step. In details, about 0.4 g plant powder was weighted into Teflon tubes. Fraction 1, the powders were extracted in 10 mL ethanol (80%, v/v) for 20 h and then centrifuged at 14,480 × *g* for 10 min (RC 6+, Thereo, USA). The supernatant liquid were transferred into beakers, while the residues were repeated with another 10 mL ethanol. The supernatant liquids were mixed, which was defined as the ethanol-extractable fraction (F_{eth}, including nitrates, chlorides and amino acid salts), and evaporated to almost dry before digested with 2 mL HNO_3. The solutions were evaporated and digested twice, then determined with AAS. Fraction 2, the residues were subsequently extracted with 10 mL 0.6 mol L^{-1} HCl for 20 h and then followed the procedure as Fraction 1. The supernatant liquid, defining as the acid-extractable fraction (F_{acid}, including phosphates, oxalates, and fraction bounding to protein), were digested, evaporated and determined as F_{eth}. Fraction 3, the centrifuged residues of Fraction 2 was digested as plant tissue in Section 2.3, which was the residue fraction (F_{res}). Prior to metal analyses, all digestion solutions were diluted to 25 mL with 2% HNO_3.

2.4. Quality control

For quality control, certified soil of GBW07429, GBW07415 and certified plant of GBW07605 provided by China National Center for Standard Reference Materials were used to ensure the reliability of the results. The standard solutions of metals (1000 mg L^{-1}; National Institute of Metrology, China) for the calibration of atomic absorption spectroscopy determinations were diluted to appropriate concentrations using 2% HNO_3. Reagents used in the experiment were guaranteed or