Ecotoxicology and Environmental Safety 159 (2018) 172–181

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Effects of cadmium and copper mixtures to carrot and pakchoi under greenhouse cultivation condition

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ARTICLE INFO

Keywords:
Cadmium
Copper
Soil
Carrot
Pakchoi

ABSTRACT

A pot experiment was undertaken to investigate the effects of Cd and Cu mixtures to growth and nutrients (sugar, carotene or vitamin C) of carrot and pakchoi under greenhouse cultivation condition. The study included: (a) physical-chemical properties of soil and soil animals in response to Cd and Cu stress; (b) bioaccumulation of heavy metals, length, biomass, contents of sugar and carotene (vitamin C) of carrot and pakchoi; (c) estimation the effects of Cd and Cu mixtures by multivariate regression analysis. The results implied that heavy metals impacted negative influence on soil animals' abundance. The metals contents in plants increased obviously with Cd and Cu contamination in soil. The biomass production and nutrients declined with Cd and Cu contents increasing. Cd (20 mg kg^{-1}) treatment caused maximum reduction of sugar content (45.29%) in carrot root; maximum reduction in carotene content (75.73%) in carrot, 75.1% sugar content reduction and 70.58% vitamin C content reduction in pakchoi shoots were observed with addition of Cd (20 mg kg^{-1}) and Cu (400 mg kg^{-1}) mixture. The results of multivariate regression analysis indicated that combination of Cd and Cu exerts negative effects to both carrot and pakchoi, and both growth and nutrients were negatively correlated with metals concentrations. It is concluded that the Cd and Cu mixtures caused toxic damage to vegetable plants as Cd and Cu gradient concentrations increased.

1. Introduction

Heavy metals are one of the most important inorganic contaminants and they are a growing concern throughout the world because their severe toxicity, non-biodegradation and high mobility and availability, which may impose potential threat to ecological environment and human health (Shukla et al., 2011). In the latest decades, heavy metals pollution in agriculture became a severe issue due to anthropogenic activities, such as mining or industrial activities, wastewater irrigation, improper use of heavy-metal-enriched fertilizers and pesticides (Baig and Kazi, 2012; Islam et al., 2015; Shukla et al., 2011).

Sharma et al. (2008) found that Cd and Cu impose a health risk on local residents. Cd is widespread in nature, but nonessential for organism. It is one of the greatest environmental concerns due to its high bioavailability and easily entry into the food chain, which leads to sever toxicity to organisms (Nazar et al., 2012). Cu is essential micronutrient in trace quantities, but it can be a potential toxicant at high concentrations. It can cause morphological, anatomical and physiological changes in plants, decreasing both food productivity and quality (Gallego et al., 2012; Qian et al., 2009). The mixtures of Cd and Cu cannot be ignored especially in Northeast China, because the pollution of Cd and Cu in soil is becoming more and more serious due to the excessive usage of agrochemicals (Liu et al., 2013a,2013b; Mico et al., 2006).

Fresh vegetables are essential for human healthy diet because they contain essential nutrients such as proteins, vitamins, carbohydrates, minerals and trace elements (Adenusi et al., 2015; Amin et al., 2013; Ivey et al., 2012; Gebrekidan et al., 2013). However, vegetables are vulnerable to Cd and Cu and easily accumulate them in their roots, stems and leave, then delivered into human body via dietary consumption of vegetables (Roy and Mcdonald, 2015; Sun et al., 2013; Weldegebriel et al., 2012). Therefore, consumption of vegetables contaminated by heavy metals may pose risks to human health (Trebolazabala et al., 2017). Many studies showed that heavy metals caused chronic poisoning and pernicious effect on the normal metabolism, human internal organs dysfunction and bring irreversible damage to our central nervous system (Zheng et al., 2010; Zhu et al., 2016). Nowadays, consumers pay more attention to food safety (Cheng et al., 2016; Huang et al., 2014).

In recent years, subjects about the effect of single heavy metal to

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https://doi.org/10.1016/j.ecoenv.2018.04.060
Received 7 February 2018; Received in revised form 23 April 2018; Accepted 26 April 2018
Available online 21 May 2018
0147-6513/

vegetables have been fully investigated (Albarracín et al., 2010; Jinadasa et al., 2016; Su et al., 2012; Xin et al., 2013). There are, however, few reports in the literatures on combined pollution of Cd and Cu in Northeast China. Nonetheless, two or more kinds of heavy metals elements usually co-exist in many cases of soil pollution, namely heavy metals combined pollution (Keltjens et al., 1998; Fu et al., 2009). Therefore, it is crucial to investigate the effects of Cd-Cu combined pollution on vegetables.

In the present study, carrot and pakchoi, widely consumed in China, were selected as tested vegetables. The pot experiment was conducted to investigate the effect of Cd and Cu mixtures on the physical-chemical properties of soil and the population characteristic of soil animals as well as biomass production and nutrients content of carrot and pakchoi. Generally, this study aims to investigate mechanisms of effect of Cd and Cu mixtures contamination on vegetables; to provide theoretical basis for soil quality assessment and vegetables cultivation with heavy metals mixtures contamination.

2. Material and methods

2.1. Experimental design

The greenhouse for this experiment was situated in Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun, China (44°0′11″N, 125°23′58″E). The temperature in the greenhouse was in the range of 18–30 °C and the air humidity varied between 55% and 80% during the study period.

Clay loam soil for vegetable cultivation was derived from farmland of Changchun, China. The physicochemical properties of the soil were: pH 7.45, organic matter 2.43%, total N content 1.05 $g\,kg^{-1}$, total P content 0.58 $g\,kg^{-1}$ and the contents of Cd and Cu were 0.09 and 19.64 $mg\,kg^{-1}$, respectively. After the soil sample was air-dried and passed through 2 mm sieve, and then the amount of 80 kg soil was put into each pot for carrot and pakchoi cultivation. Cd was added as Cd $(NO_3)_2{\cdot}4(H_2O)$, and Cu as Cu $(NO_3)_2{\cdot}3(H_2O)$. Sixteen treatments were designed as presented in Table 1. There were 6 replicates for each treatment. The pot sizes were 50 cm (length) × 30 cm (width) × 40 cm (depth). To ensure normal growth of vegetables, fertilization added per kg soil was 0.75 g NH_4NO_3 and 0.44 g K_2SO_4, respectively. The carrot and pakchoi were harvested after the 120-day and 60-day growth period, respectively. All samples were thoroughly washed with deionized water. Some fresh vegetable samples were used to measure length and quality analysis (carotene, sugar, vitamin C) immediately, and the remainder was oven-dried at 70–80 °C to a constant weight for dry biomass and further mineral analysis. Soil samples were taken for determining pH, organic matter, concentrations of Cd and Cu and amounts of soil animals.

2.2. Soil analysis

2.2.1. Physic-chemical properties

Soil pH value was determined (soil-water ratio, 1:2.5) by potentiometric (pHS-3B, Leici, Shanghai) (Sun et al., 2013) and organic matter content was determined by Walkley-Black method (Gan et al., 2017).

Digestion mixture (10 ml) which contained H_2SO_4 and SeO_2 was added to a 150 ml conical flask with 1 g soil sample. Salicylic acid was also added to include the nitrates and nitrites. Digestion was conducted till the soil color changed to white. Total N in the digest was measured by using micro-Kjeldahl method (Mani et al., 2012).

A known weighed soil (2 g) was taken with 4 ml $HClO_4$ (70%) into a 50 ml beaker covered with watch glass and put on a hot plate and digested till the soil color changed to white. Ten ml HNO_3 was added to the filtrate solution. Ammonia was added to saturate the solution. Then 30 ml standard ammonium molybdate solution was added in the solution for extraction of the total P content in soil.

2.2.2. Heavy metals in soil

The soil samples were air-dried at ambient temperature, crushed, and passed through a 0.149-mm nylon sieve and then digested in triplicate with the mixture of HF, $HClO_4$ and HNO_3 for analysis of Cd and Cu total contents (Liu et al., 2013a,2013b). The total concentration of Cd in the soils was determined by graphite furnace atomic absorption spectroscopy (GFAAS; AA-6300C, Shimadzu, Japan). The total concentration of Cu in the soil samples was determined by flame (air-acetylene) atomic absorption spectroscopy (FAAS; AA-6300C, Shimadzu, Japan).

The analysis of available heavy metals in soil was performed according to the procedure described by Zeng et al. (2011). Available Cd and Cu were extracted by a mixture containing 0.05 $mol\,L^{-1}$ ethylene-diamine-tetra-acetic acid disodium (EDTA-Na_2), 0.01 $mol\,L^{-1}$ $CaCl_2$ and 0.1 $mol\,L^{-1}$ tri-ethanolamine (TEA). Briefly, 10 ml EDTA-$CaCl_2$-TEA mixture was added to polypropylene tubes with 5 g soil sample (soil mixture ratio 1:2, at pH = 7.0). The polypropylene tubes were shaken on a reciprocal shaker at 150 rpm for 3 h, the suspensions were centrifuged at 3000 rpm for 20 min and then filtered using 0.45 mm membrane filter. Extracted Cd and Cu concentrations in filtrates were measured by graphite furnace atomic absorption spectroscopy (GFAAS; AA-6300C, Shimadzu, Japan) and flame atomic absorption spectroscopy (FAAS; AA-6300C, Shimadzu, Japan).

2.2.3. Extraction of soil animals

Three soil cores (10 cm (length) × 10 cm (width) × 10 cm (depth)) obtained from each pot referring to the method described by Marx et al. (2016) and immediately returned to the laboratory for extraction of soil animals. Soil animals were extracted from the cores used Tullgren funnels with 25 W lights as light and heat sources for 72 h according to the method of Culik et al. (2002). Soil samples were placed on 2 mm wire mesh in the funnels approximately 10 cm below the bulbs. Collection containers (100 ml) with approximately 50 ml 70% ethanol were attached below the funnels for subsequent identification.

2.3. Vegetable analysis

2.3.1. Heavy metals in vegetables

Roots and shoots of dried carrot and pakchoi were digested with HNO_3-$HClO_4$ referencing the method of Gebrekidan et al. (2013). The concentrations of Cd in the vegetable samples were determined by graphite furnace atomic absorption spectroscopy (GFAAS; AA-6300C,

Table 1
pH value and organic matter (%) of tested soil.

items	Cd0[b]	Cd0.2	Cd2.0	Cd20
Cu0[a]	7.45 ± 0.06–(2.43 ± 0.12)[*]	7.42 ± 0.29–(2.39 ± 0.09)	7.40 ± 0.09–(2.08 ± 0.14)	7.38 ± 0.20–(2.04 ± 0.05)
Cu20	7.40 ± 0.12–(2.54 ± 0.19)	7.51 ± 0.14–(2.51 ± 0.12)	7.52 ± 0.21–(2.22 ± 0.29)	7.12 ± 0.28–(2.51 ± 0.06)
Cu200	7.36 ± 0.06–(2.47 ± 0.23)	7.34 ± 0.25–(2.38 ± 0.12)	7.27 ± 0.11–(2.26 ± 0.19)	7.35 ± 0.19–(2.08 ± 0.04)
Cu400	7.41 ± 0.06–(2.23 ± 0.28)	7.54 ± 0.04–(2.12 ± 0.11)	7.46 ± 0.26–(2.36 ± 0.12)	7.39 ± 0.04–(2.20 ± 0.06)

[a] Cu added levels in soil of different treatments.
[b] Cd added levels in soil of different treatments.
[*] pH value–(organic matter).