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Feasibility of lettuce cultivation in sophoroliplid-enhanced washed soil originally polluted with Cd, antibiotics, and antibiotic-resistant genes

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ABSTRACT

Vegetable cultivation in soils polluted with heavy metals, antibiotics and a high abundance of antibiotic-resistance genes (ARGs) can seriously threaten human health through the food chain. Therefore, novel techniques that not only remediate soil, but also ensure food security are urgently required. In the present study, two successive washings with 20 g L^{-1} of sophoroliplid solution plus ultrasonication (35 kHz) were effective in extracting 71.2% Cd, 88.2% tetracycline, 96.6% sulfadiazine, and 100% roxithromycin. Simultaneously, relative abundance of ARGs (*tet*M, *tet*X, *sul*I, and *sul*II) was decreased to 10^{-7}–10^{-8} (ARG copies/16S copies). Further, lettuce cultivation in the 2nd washed soil showed significant improvement in vegetable growth indices (fresh/dry weight, root surface area, chlorophyll content and soluble protein content) and a decrease in isolate counts for antibiotic-resistant bacterial endophytes and ARG abundance in lettuce tissues. This combined cleanup strategy provides an environmentally friendly technology for ensuring vegetable security in washed soils.

1. Introduction

Application of livestock manure, an effective and natural organic fertilizer, to farmland soil has been accepted as a common agronomic method to promote the growth of vegetables and crops (Wang et al., 2015). However, with the rapid development of the livestock industry worldwide, large amounts of livestock feces containing antibiotic drug compounds and heavy metal additives have gradually entered arable soils (Cheng et al., 2013). This not only resulted in the direct input of contaminants in the soil but also posed a huge natural selection pressure on the soil microbial community, which facilitated the proliferation and occupation of a dominant niche for antibiotic-resistant bacteria (ARB) (Peng et al., 2015). At the same time, antibiotic presence can also induce abnormal expression of soil antibiotic resistance genes (ARGs), which pose a high risk of being integrated into the DNA of human pathogenic bacteria (Heuer et al., 2011). As a result, ARGs have been considered as novel biological pollutants (Ji et al., 2012). Heavy metals in the soil have been demonstrated to further increase the over-expression of ARGs (Zhu et al., 2013). Therefore, planting vegetables in such soils would result in a serious security threat to human health via the food chain. Thus, it is crucial to develop a novel technology for remediating antibiotics and heavy metals in mixed contaminated soils rich in ARB and mitigating soil ARG abundance.

Soil washing is an effective technique for removing both organic and inorganic contaminants (Cao et al., 2015; Kulikowska et al., 2015). However, the effect of this technique on the removal of antibiotics and metals from mixed contaminated soil with abundant ARB and ARGs has not been investigated yet. Moreover, the feasibility of vegetable cultivation in washed soil and vegetable security need to be studied.

In this study, we used a sophorolipid as an environmentally friendly eluent to perform remediation via soil washing. The sophorolipid is a type of green biosurfactant. It is composed of a hydrophilic sophorose unit and a hydrophobic fatty acid portion, with the critical micelle concentration and hydrophilic–lipophilic balance of 40–100 mg L^{-1} and 10^{-13}, respectively (Mousavi et al., 2015). This study was designed to (i) investigate the effect of eluent concentration, ultrasonication, and temperature on the

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removal of antibiotics, heavy metals, and ARGs from the soil and (ii) explore vegetable quality and security risk of lettuce cultivation in the washed soil. The results can provide key information on the remediation and management of vegetable cultivation in farmland soils contaminated with antibiotics, heavy metals, ARB, and ARGs.

2. Materials and methods

2.1. Soil sampling and preparation

Test soil samples were collected in December, 2014 at Zhu jiashan (ZJ) dairy farm in the suburban area of Nanjing, Eastern China (31°99′90′ N, 119°10′4′ E). The dairy farm is located in the vicinity of Nanjing Chemical Industry Park. There is a high risk of various pollutions from antibiotics and heavy metals in the soil over 20 years (1994–2014). Topsoil samples (0–10 cm, about 50 kg in total) were collected from 10 locations around the farm. All soil samples were homogenized, grounded and passed through a 2.0 mm sieve. A portion of each sample was air dried for 7 d at room temperature, the residual subsample was kept moist and stored in a sealed glass container at 4 °C prior to analysis. The soil physical–chemical properties were shown in Supplementary materials (Table S1). According to preliminary surveys, the mixed contaminants in soil mainly existed as cadmium (Cd), tetracycline (TC), sulfadiazine (SD) and roxithromycin (ROX).

2.2. Soil washing setup

One kilogram of initial polluted soil was added into an 8 L stainless steel vessel, to which 5 L of different concentrations of sophorolipid solution was added, and the vessel was placed on a stirring engine. Four treatments were designed (Supplementary materials, Table S2), (1) S1: sophorolipid+25 °C, (2) S2: sophorolipid+25 °C+ultrasonication, (3) S3: sophorolipid+50 °C, (4) S4: sophorolipid+50 °C+ultrasonication. Each treatment was replicated three times. The testing concentrations of sophorolipid solution (w/v) were 0–50 g L^{-1}. The vessel was stirred at 25 ± 2 °C or 50 ± 2 °C at 50 rpm for 60 min. Then, ultrasonication was generated by four series connection ultrasonic homogenizer welded on the outer wall of the vessel. Each generator was operated at 35 kHz for 15 min. After the washing step, soil was separated from the supernatant by centrifugation (2000 rpm for 20 min) for subsequent analysis.

For the purpose of prompting the mixed pollutant removal efficacy and reducing the dosage of the washing solvent, optimized washing parameters combination was employed in the following successive washing procedure (from 1 to 3 cycles). Successive washed soils were then combined and air-dried for the vegetable cultivation experiments.

2.3. Lettuce cultivation

Based on the preliminary investigation, soils after two successive washing were proved to be the lowest in the residual mixed pollutant, and were used for the lettuce cultivation trials. Soil microcosms were set up using a series of polyvinyl chloride rectangular pots (lower length, 45 cm; lower width, 30 cm; height, 25 cm), each containing 5000 g of air-dried soil. The treatments were designated as follows: (1) T1: Lettuce cultivation in initial polluted soil, and (2) T2: Lettuce cultivation in 2nd washed soil. Each treatment was triplicated three times.

Lettuce seeds obtained from the Jiangsu Academy of Agricultural Sciences were germinated, and seedlings were grown in moist perlite for 10 d, after which three seedlings of uniform size were transplanted to each pot. The pots were set in a greenhouse with an average temperature of 25 ± 2 °C and a 12 h light/12 h dark photoperiod rotation for 100 days. Every 10 days, approximately 10 g of soil was collected by taking five random soil samples up to a depth of 10 cm from each pot. The samples were combined to give one composite sample per pot (50 g). At the end of the trial, three plants were harvested and carefully rinsed with nanopure water. After 100 days of growth, all the plant and soil samples were put into plastic bags, and stored at 4 °C prior to the analysis. The weight of lettuce tissues, total root surface area, root activity, chlorophyll content, and soluble protein content were determined following the methods described by Ahmed et al. (2015).

2.4. Antibiotics and heavy metal analysis

The extraction for soil TC, SD, and ROX followed the methods described by Sun et al. (2015). Changes of different fractions of soil antibiotics (water-soluble, exchangeable, loosely bound, and tightly bound fractions) were analyzed according to Zhou et al. (2013). 1.0 g of soil sample was placed in a 50-mL centrifuge tube and extracted with 40 mL of extract buffer (H_2O-, $CaCl_2$-, and McIlvaine-). All the tubes were sonicated for 20 min and centrifuged at 5000 rpm at 4 °C for 4 min. The supernatants were combined and filtered (pore size 0.45 μm, PVDF membrane filter). The liquid was purified and concentrated using a 60-mg Oasis HLB column from Waters (Milford, MA). Final extracts were analyzed using HPLC-MS (Agilent 1260 series, Agilent Technologies, CA, USA) (Sun et al., 2015). Antibiotics concentrations in lettuce tissues were measured following the methods of Zhang et al. (2015). The lettuce tissues (0.5 g) were freeze-dried and ground into 80 mesh. A mixed solution (10 mL), containing phosphate buffer, acetonitrile, and lettuce tissues, was added into each centrifuge tube. The tubes were shaken in a shaker at 250 rpm for 20 min, sonicated for 10 min, and then centrifuged at 7000 × *g* for 10 min. The samples were extracted using Oasis HLB extraction cartridges (6.0 mL, 200 mg). The cartridge was rinsed with 0.1% methanoic acid solution (6.0 mL) containing 5.0 mmol L^{-1} acetic acid, and then was eluted with methanol (6.0 mL). The fraction was redissolved in 10% methanol to the volume of 1.0 mL for subsequent HPLC-MS analysis. Freshly prepared antibiotics standard solutions were used to calibrate HPLC. SA Quantification was using external standard calibration, and the correlation coefficient was no less than 0.995 for each antibiotic. In this study, the whole lettuce tissue was divided into the underground (roots) and aboveground (leaves) tissues. All the antibiotic concentrations were analyzed using HPLC-MS with electron spray ionization in a multiple reaction monitoring mode (Hou et al., 2015). The average recoveries for TC, SD, and ROX were between 94.2 ± 2.6% and 106.1 ± 4.2%.

The total concentration of Cd was measured by digesting 2 g soil samples using 50 mL HCL–HNO_3–$HClO_4$ (4:2:1, v/v/v) and was quantified with a Thermo flame Atomic Absorption Spectrophotometer (Ji et al., 2012). Consecutive extraction method developed by the European Communities Bureau of Reference were operated on different test soil samples to determine the heavy metal redistribution among operationally defined pools. The average recoveries for Cd were between 95.8 ± 2.3% and 102.3 ± 1.2%.

2.5. Identification of antibiotic-resistant bacterial endophyte in lettuce tissues

Lettuce roots and leaves were washed thoroughly with adequate deionized water to eliminate adhering particles and surface microbes. The lettuce tissues surface decontamination was carried out following the procedure described by Bhore et al. (Bhore et al., 2010). Then the small pieces of tissue (1 cm^2) were incubated on