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Effects of soil texture and drought stress on the uptake of antibiotics and the internalization of *Salmonella* in lettuce following wastewater irrigation

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A B S T R A C T

Treated wastewater is expected to be increasingly used as an alternative source of irrigation water in areas facing fresh water scarcity. Understanding the behaviors of contaminants from wastewater in soil and plants following irrigation is critical to assess and manage the risks associated with wastewater irrigation. The objective of this study was to evaluate the effects of soil texture and drought stress on the uptake of antibiotics and the internalization of human pathogens into lettuce through root uptake following wastewater irrigation. Lettuce grown in three soils with variability in soil texture (loam, sandy loam, and sand) and under different levels of water stress (no drought control, mild drought, and severe drought) were irrigated with synthetic wastewater containing three antibiotics (sulfamethoxazole, lincomycin and oxytetracycline) and one *Salmonella* strain a single time prior to harvest. Antibiotic uptake in lettuce was compound-specific and generally low. Only sulfamethoxazole was detected in lettuce with increasing uptake corresponding to increasing sand content in soil. Increased drought stress resulted in increased uptake of lincomycin and decreased uptake of oxytetracycline and sulfamethoxazole. The internalization of *Salmonella* was highly dependent on the concentration of the pathogen in irrigation water. Irrigation water containing 5 Log CFU/mL *Salmonella* resulted in limited incidence of internalization. When irrigation water contained 8 Log CFU/mL *Salmonella*, the internalization frequency was significantly higher in lettuce grown in sand than in loam ($p = 0.009$), and was significantly higher in lettuce exposed to severe drought than in unstressed lettuce ($p = 0.049$). This work demonstrated how environmental factors affected the risk of contaminant uptake by food crops following wastewater irrigation.

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1. Introduction

Agricultural irrigation with wastewater is a common practice in arid areas around the world, and is expected to be adopted by more regions due to growing food demands and increasing fresh water scarcity (United Nations Environment Programme, 2002). Despite the social and economic benefits of wastewater irrigation, there are potential risks of food contamination from the microconstituents in wastewater, such as the bioaccumulation of human pathogens (e.g. *Escherichia coli* O157:H7 and *Salmonella* spp.) and antibiotics in food crops through root uptake (Mitra et al., 2009; Sabourin et al., 2012; Sallach et al., 2015b; Wu et al., 2014).

Antibiotics prescribed to human and livestock may occur in urine and feces due to incomplete absorption (Kumar et al., 2005). Concentrations of antibiotics have been detected in the mg/L levels in agricultural wastewater (Aga et al., 2003; Campagnolo et al., 2002; Zilles et al., 2005) and low μg/L levels in municipal wastewater effluents (Hirsch et al., 1999; Joss et al., 2005; Karthikeyan

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and Meyer, 2006). The concentrations of bacterial pathogens are highly variable in wastewater. For example, *Salmonella* population typically ranges from 2 to 4 log cells/100 mL in municipal wastewater (Hu and Gibbs, 1995; National Research Council, 1998), but as high as 5 to 9 log cells/100 mL has been documented (Jimenez et al., 1999). Although more than 70% *Salmonella* can be removed by secondary treatment (National Research Council, 1998), significant regrowth in recycled wastewater distribution system can occur (Westrell et al., 2004).

The accumulation of antibiotics and pathogens in plants following wastewater irrigation can be affected by various environmental factors. Soil texture is an important soil property based on the percentage of sand, silt, and clay. Soil with the finest texture is categorized as clay (containing $\geq$ 40% clay), while soil with the coarsest texture is categorized as sand (containing $\geq$ 85% sand) (USDA, 1987). In the major lettuce production regions in the U.S., which includes Yuma county in Arizona and Imperial county in California, the soil texture varies from the finest clay to the coarsest sand (Barmore, 1980). Although lettuce can grow in a wide range of soil types, light textured soils like sandy loam soils are preferred because they provide sufficient drainage and nutrients (Westerfield, 2012).

Few studies have examined the influence of soil properties on the plant uptake of antibiotics. One study reported that the concentration of the seizure treatment drug carbamazepine in cucumber fruits decreased with increasing soil organic matter (SOM) in soil (Shenker et al., 2011), because SOM affects the bioavailability of organic contaminants in soil (Hung et al., 2009). Organic matter is also important in determining contaminant concentrations in the interstitial water in soil, which in turn affects the equilibrium concentrations of contaminants in plant (Carter et al., 2014; Chiou et al., 2001). Another study found that the extractability of organic contaminants phenanthrene and atrazine from soil was a function of soil texture and soil organic carbon (Chung and Alexander, 2002). Based on these results it's plausible to expect that, in soils with low SOM, the uptake of antibiotics by plants would also be affected by soil texture.

Soil texture can also affect the fate and mobility of human pathogens in soil. Clay soil can reportedly better support microbial survival and growth than sandy soil due to its ability to hold organic matter and water (Holley et al., 2006; Nicholson et al., 2005). On the other hand, bacteria are less mobile in finer soil as a result of more efficient filtration and adsorption by clay (Jamieson et al., 2002). Although soils of different textures were used in studying pathogen contamination of plants (Franz et al., 2005; Natvig et al., 2002), the effects of soil texture on the internalization of human pathogens into food crops have not been systematically investigated.

Drought is another environment condition worth investigating as it occurs regularly in acrid areas and is projected to become more severe and widespread in the coming decades (Dai, 2013). Unfortunately, little is known about the effects of drought stress on the uptake of antibiotics and the internalization of pathogens in plant. The uptake ability of plants for nutrients can fully recover 2 days after drought (Buljovcic and Engels, 2001), and different plant species exhibited different abilities to recover (Steinemann et al., 2015; Xu et al., 2010). Under normal conditions the uptake of antibiotics in plants is very limited (Wu et al., 2014), however, it is unknown how drought stress may affect the uptake. On the other hand, it is known that drought can lower plants' ability to resist plant pathogens (Garrett et al., 2006), but it is unclear if similar effect happens to human pathogens. Contradictory observations have been reported on how drought may affect the internalization of human pathogen into plants. Zhang and co-workers inoculated soil with *E. coli* O157:H7 prior to exposing lettuce plants to drought stress and found no internalized *E. coli* in leaves (Zhang et al., 2009b). In contrast, Ge and co-workers found that drought significantly increased the internalization concentration of *Salmonella enterica* serovar Typhimurium into lettuce (Ge et al., 2012).

The objective of this study was to evaluate the effects of soil texture and drought stress on the uptake of antibiotics and the internalization of human pathogens into lettuce through root uptake following wastewater irrigation. Sulfamethoxazole, lincomycin, and oxytetracycline were used as model antibiotics to represent three chemically unique classes of antibiotics: sulfonamide, lincosamide, and tetracycline. A *S. enterica* strain was used as a model human pathogen in this study. Lettuce plants were exposed to wastewater containing antibiotics and *Salmonella* in one irrigation event before harvesting. Three soil textures each with little SOM and three drought levels were tested in this study. Results can be used to develop strategies to minimize the uptake of antibiotics and human pathogens by plants and to manage the potential risk associated with wastewater irrigation.

2. Materials and methods

2.1. Chemicals

Lincomycin, roxithromycin, doxycycline hyclate, and demeclocycline hydrochloride were purchased from Sigma–Aldrich (St. Louis, MO). Sulfamethoxazole and oxytetracycline were obtained from MP Biomedicals, LLC (Solon, OH). ^{13}C-Sulfamethazine was purchased from Cambridge Isotope Laboratories (Andover, MA). Standard stock solutions were prepared with HPLC grade methanol and stored in dark at −20 °C. Surrogate and internal standard spiking solutions were prepared in methanol biannually at the UNL Water Sciences Laboratory. Calibration standards (0.1–5 ng/μL) were prepared prior to each analysis in 3:1 (v/v) solution of NanoPure water (Barnstead, Dubuque, IA) and methanol.

2.2. Bacterial strain

A *S. enterica* serovar Infantis strain was obtained from Dr. Lisa Durso of the USDA Agricultural Research Service. This *Salmonella* strain was isolated from the soil in a feedlot that had been amended with cattle manure. *Salmonella* was cultured in Luria broth (LB) at 37 °C for 17 h and harvested at the mid-stationary phase. Cells were collected by centrifuging at 6000× *g* at 4 °C for 10 min. The cell pellet was re-suspended in phosphate buffered saline (PBS), and then diluted to 5 and 8 log CFU/mL in de-chlorinated tap water prior to irrigation.

2.3. Uptake experiment with various soil textures

Three soils with distinctive textures were prepared by mixing sand into Sharpsburg silt clay at the weight ratios of 75:25, 50:50 and 25:75. The properties of the soils were characterized at Midwest Laboratories (Omaha, NE) and reported in Table 1. Seeds of leafy lettuce (*Lactuca sativum*) cultivar Green Salad Bowl, common in organic lettuce production, were germinated and grown in each soil in a BSL-2 greenhouse with 16 h of light every day at 15–18 °C. Eight seeds were initially planted in each $17 \times 12 \times 7$ cm^3 (L × W × H) pot, and six pots were placed in a tray. Lettuce seedlings were thinned to four plants per pot upon germination, and further thinned to 1 plant per pot at week 4. Trays were sub-irrigated with clean, de-chlorinated tap water for 6 weeks until the final irrigation before harvest. The amount of water used in each irrigation event was determined based on the field capacity of each soil texture. Approximately 3 days prior to harvest, each tray was sub-irrigated with 1 L de-chlorinated water (control), low-, or high-strength synthetic wastewater. The low-strength

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