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Uptake of antibiotics from irrigation water by plants

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HIGHLIGHTS

- The capacity of carrot and lettuce to take up antibiotics was investigated.
- Human risk to antibiotics through vegetables irrigated with contaminated water was low.
- Uptake of antibiotics followed a dose–response effect.
- Carrot showed higher average concentration of antibiotics than lettuce samples and was significant.
- Sub-MIC concentrations of antibiotics via vegetables consumed in diet could promote antibiotic resistance.

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ABSTRACT

The capacity of carrot (*Daucus corota* L.) and lettuce (*Lactuca sativa* L.), two plants that are usually eaten raw, to uptake tetracycline and amoxicillin (two commonly used antibiotics) from irrigated water was investigated in order to assess the indirect human exposure to antibiotics through consumption of uncooked vegetables. Antibiotics in potted plants that had been irrigated with known concentrations of the antibiotics were extracted using accelerated solvent extraction and analyzed on a liquid chromatograph-tandem mass spectrometer. The plants absorbed the antibiotics from water in all tested concentrations of 0.1–15 mg L^{-1}. Tetracycline was detected in all plant samples, at concentrations ranging from 4.4 to 28.3 ng/g in lettuce and 12.0–36.8 ng g^{-1} fresh weight in carrots. Amoxicillin showed absorption with concentrations ranging from 13.7 ng g^{-1} to 45.2 ng g^{-1} for the plant samples. The mean concentration of amoxicillin (27.1 ng g^{-1}) in all the samples was significantly higher ($p = 0.04$) than that of tetracycline (20.2 ng g^{-1}) indicating higher uptake of amoxicillin than tetracycline. This suggests that the low antibiotic concentrations found in plants could be important for causing antibiotics resistance when these levels are consumed.

1. Introduction

Antibiotic residues are widespread in the environment and are well documented in various aquatic compartments including municipal sewage (Castiglioni et al., 2006; Gao et al., 2012; Lillenberg et al., 2010; Osorio et al., 2012), hospital sewage (Chang et al., 2010; Duong et al., 2008), groundwater (Hirsch et al., 1999) and surface water (Kolpin et al., 2002; Yan et al., 2013), usually at concentrations in the ng L^{-1} to a few μg L^{-1} range (Zuccato et al., 2010). Tetracycline has been reported in concentrations up to 110 ng L^{-1} in surface water (Kolpin et al., 2002), whiles amoxicillin has been found in levels up to 120 ng L^{-1} in sewage treatment plant effluents (Andreozzi et al., 2004). In low and middle-income countries, antibiotics are widely available to the public, from a variety of sources, including hospitals and pharmacies, licensed medicine stalls and drugstores, roadside stalls and peddlers (Lerbech et al., 2014; Senah, 1997; Wolf-Gould et al., 1991). Despite prohibitive legislations, antibiotics can be purchased without prescription (Okeke et al., 2007; Radyowijati and Haak, 2003; Seiter and Gyansa-lutterodt, 2009). This widespread availability has led to inappropriate use by patients and healthcare providers (Adu-Sarkodie, 1997; Radyowijati and Haak, 2003; Wolf-Gould et al., 1991). The occurrence and implications

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of antibiotic residues in the environment is an emerging concern. Attention is currently being giving to low doses of drug residues that may occur in foods (Boxall et al., 2006; Herklotz et al., 2010; Kumar et al., 2005). Although human health implications due to antibiotic residues in food crops are largely unknown, several potential adverse impacts including allergic/toxic reactions, chronic toxic effects as a result of prolonged low-level exposure (Phillips et al., 2004; Sarmah et al., 2006), development and spread of antibiotic-resistant bacteria (Gullberg et al., 2011; Kim and Aga, 2007; van den Bogaard, 2000), and disruption of digestive system function (Bedford, 2000; Schuijt et al., 2013) have been speculated.

Soil acts as a sink for the pharmaceuticals released into the environment. At present, scientific understanding of the behaviour of antibiotics in soils and plants is very sketchy and highly speculative. Evaluation of data on biodegradation is very difficult as kinetics are mostly unknown (Winker, 2010). A couple of studies have recently demonstrated that plants can take up pharmaceutical compounds from the growth media via their roots (Ahmed et al., 2015; Boxall et al., 2006; Chitescu et al., 2013; Dolliver et al., 2007; Herklotz et al., 2010; Kumar et al., 2005). Pot experiment studies have shown that plants usually take up less than 2% of the pharmaceuticals applied to soil (Dolliver et al., 2007; Kumar et al., 2005). The fate and transport of antibiotics in the environment depend on the underlying physical properties of the compounds such as water solubility, lipophilicity, volatility and sorption potential. Sorption of antibiotics in the soil can reduce their mobility, reactivity, and bioavailability for microbial degradation (Hatzinger and Alexander, 1997). Soil properties such as organic carbon content, ionic strength, clay content, texture, and pH can alter sorption mechanisms involved, and the extent of sorption (OECD, 2000).

The objective of the present study was to evaluate the extent to which carrot (*Daucus corota* L.) and lettuce (*Lactuca sativa* L.) can absorb tetracycline and amoxicillin from soils in order to estimate human health risk due the consumption of vegetables contaminated with antibiotics. Tetracyclines and penicillins (amoxicillin) are among the most used antibiotics in Ghana (Annan-Prah et al., 2012; Sasu et al., 2012; Tagoe and Attah, 2010; Turkson, 2008). The vegetables studied are extensively produced by urban vegetable farmers in Ghana and they are mostly eaten raw or sometimes with little cooking (Drechsel and Keraita, 2014).

2. Methodology

2.1. Chemicals and reagents

Tetracycline hydrochloride (CAS #: 60-54-8, >96% pure) was obtained from Sigma-Aldrich (Steinheim, Germany) while amoxicillin trihydrate (CAS #: 267-87-780, 98% pure) was obtained from Fluka (Steinheim, Germany). The deuterated standard (internal standard), d_3-trimethoprim, was purchased from Qmx Laboratories (Thaxted, UK). Methanol (HPLC grade) was obtained from Lab-Scan (Gliwice, Poland), formic acid (98–100% pure, Ph Eur) was from Merck KGaA (Darmstadt, Germany). Milli-Q water was produced in-house with a Milli-Q water gradient system (Millipore, Bedford, Massachusetts, USA). Stock solutions were prepared with methanol and stored in freezer at −18 °C.

2.2. Test soils

Two test soils were used. The first test soil (Soil 1) was sterilized soil obtained from the Department of Horticulture, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. The second test soil (Soil 2) was collected from Karikari Vegetable Farmland (GPS point: 1.57,755 E, 6.65,638 N) in Kumasi, Ghana. Soils were air-dried and passed through a 2 mm screen and mixed thoroughly prior to characterization and use in the uptake studies. Table 1, summarizes the properties of the test soils. The soil pHs were 7.01 and 7.25 for Soil 1 and Soil 2 respectively. Taxonomic classification of the soil samples were loamy fine sand for Soil 1 and silty clay loam for Soil 2. The % clay content was 7.36 for Soil 1 and 26.5 for Soil 2.

2.3. Uptake studies

One-week old seedlings of lettuce and seeds of carrot (16 each) were planted into 4 kg aliquots of soil placed in porous plastic pots (15 cm diameter × 14 cm deep). The plants were grown in a greenhouse under controlled conditions: 50% relative humidity and a temperature of 31 °C during the 12:12 light: dark regime. The transplanted lettuce seedlings were watered for 3 days with tap water to get adjusted to the new soil. The carrot seeds were watered for 60 d for the seeds to germinate and the seedlings to grow to the point of forming the tubers. During the 30 d preceeding maturation and harvesting, the plants were watered twice a day with 420 mL of spiked distilled water (210 mL in the morning and 210 mL in the evening). Each pot was separately irrigated with either 0.1, 1.0, 10.0 or 15.0 mg L^{-1} of the antibiotics. The spiked water was poured directly onto the soil at the base of the plant. Four replicates were done for each of the four concentration points and for each of the antibiotics. A control batch was only irrigated with distilled water. At maturation (40 d for lettuce and 90 d for carrot), all plant samples were harvested, washed with distilled water and dried on an adsorbent paper according to Dolliver et al. (2007). The plant samples were then bulked and kept in a refrigerator at −4 °C before shipping to Denmark where they were stored at −18 °C until use.

2.4. Extraction of compounds for chemical analysis

The tubers of carrot and leaves of lettuce samples separately chopped and frozen at −18 °C were freeze-dried for 48 h using a Heto FD3 lyophilisor (Heto Lab equipment, Allerød, Denmark) coupled to a roughing pump (Edwards, UK). The system was operated between 0.090 and 0.100 torr at −48 °C. The freeze-dried samples were then ground thoroughly with a sterile pestle. Pressurized liquid extraction (PLE) was performed on an Accelerated Solvent Extractor, ASE 200 from Dionex (Sunnyvale, CA, USA). A Dionex cellulose filter paper (1.98 cm diameter) was introduced inside the 11 mL extraction cell and gently located by a plunger into the cell's base to avoid sample aggregation, prevent clogging of the extraction cell and allow a greater exposure surface area and thereby improved contact between solvent and matrix. A 0.1 g aliquot of sample was blended gently in a 5 g Ottawa sand (20–30 mesh, AppliChem, Darmstadt, Germany) and placed inside the extraction cell. Another Dionex cellulose filter paper was placed above the packing and 50 μL of 0.4 μg mL^{-1} d_3–trimethoprim (internal standard) was added to the filter prior to extraction. Internal standard was spiked in all PLE extraction cells equivalent to a final amount of 20 ng to correct for any losses during sample preparation and for any variability in injection volume. The extracting solvent was 100% Milli-Q water and the extractor was operated at 70 °C with one cycle for a static period of 10 min. At the end of each extraction a total extract volume of approximately 30 mL was obtained.

The PLE extracts were cleaned up on a reversed phase solid-phase extraction (SPE) using Oasis HLB cartridges (hydrophilic-lipophilic balance, 200 mg sorbent, 30 μm, 6 cm^3) purchased from Waters Oasis (Massachusetts, USA). The SPE cartridges were conditioned with 3 mL MeOH followed by 3 mL distilled water. A 30 mL portion of extract was loaded on the SPE columns at a flow rate of 1.5 mL min^{-1} and then were washed with 3 mL of 5% MeOH