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Transfer of oxytetracycline from swine manure to three different aquatic plants: Implications for human exposure

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HIGHLIGHTS

- Shows for the first time transfer of OTC from swine manure to aquatic plants.
- Isotherms for desorption of OTC from swine manure constructed.
- Comparative bioconcentration of OTC by three contrasting aquatic plants measured.
- Per capita daily OTC uptake rates from aquatic plant consumption derived.
- ADI comparison shows pathway should not be ignored in determining human exposure.

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ABSTRACT

Little is known regarding the potential for pharmaceuticals including antibiotics to be accumulated in edible aquatic plants and enter the human food chain. This work investigates the transfer of a widely used veterinary antibiotic, oxytetracycline (OTC), from swine manure to aquatic plants by firstly characterizing desorption from swine manure to water and fitting data to both nonlinear and linear isotherms. Bioconcentration of OTC from water was then quantified with aquatic plants of contrasting morphology and growth habit *viz.* watermeal (*Wolffia globosa* Hartog and Plas), cabomba (*Cabomba caroliniana* A. Gray) and water spinach (*Ipomoea aquatica* Forsk.). Watermeal and water spinach are widely consumed in Southeast Asia. The OTC desorption and bioconcentration data were used to provide the first quantitative estimates of human exposure to OTC from a manure-water-aquatic plant route. Results show that under certain conditions (plants growing for 15 d in undiluted swine manure effluent (2% w/v solids) and an initial OTC swine manure concentration of 43 mg kg^{-1} (dry weight)), this pathway could provide a significant fraction (>48%) of the acceptable daily intake (ADI) for OTC. While effluent dilution, lower OTC manure concentrations and not all plant material consumed being contaminated would be expected to diminish the proportion of the ADI accumulated, uptake from aquatic plants should not be ignored when determining human exposure to antibiotics such as OTC.

1. Introduction

Oxytetracycline (OTC) is a member of the tetracycline family of antibiotics and widely used in swine farming (Matsui et al., 2008). Its poor absorption on oral administration results in the contamination of swine manure by unmetabolized material (Winckler and Grafe, 2001). Kumar et al. (2005a) report antibiotic concentrations in animal manures to range from trace levels up to 200 mg kg^{-1}. Liquid effluent from swine farms often contains suspended manure. While limited treatment of the effluent may take place at some facilities, OTC can still be discharged into receiving water (Xuan et al., 2010). In the environment, factors such as temperature, pH and light intensity can affect the degradation of OTC in water (Burhenne et al., 1997) and swine manure (Ratasuk et al., 2012).

Several studies have investigated the potential for tetracyclines to be taken up from the interstitial water of soil by plants. For example, Kumar et al. (2005b) reported that corn (*Zea mays* L.), green onion (*Allium cepa* L.), and cabbage (*Brassica oleracea* L.) could accumulate chlortetracycline while Boonsaner and Hawker (2010, 2012) found that OTC was uptaken by soybean (*Glycine max* (L.) Merr.) and rice (*Oryza sativa* L.).

Concern has been expressed that residual pharmaceuticals such as antibiotics can be taken up by edible plants and enter the human food supply (Tanoue et al., 2012). Potential adverse effects

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on humans due to accumulation of OTC in excess of the acceptable daily intake (ADI) include hepatotoxicity, disruption of digestive system functioning and development and spread of antibiotic-resistant bacteria (Dolliver et al., 2007; Boonsaner and Hawker, 2013). While maximum residue levels for antibiotics including OTC have been established for animal-based products there are no such data for plants (Kim et al., 2011). One reason for this is that relatively little is known of the potential and extent of uptake by plants (Cropp et al., 2010). Work by Boxall et al. (2006) showed that a terrestrial uptake route for crops via interstitial water in soil and manure-amended soil for veterinary medicines could account for up to 10% of the ADI for some compounds. The situation with aquatic plants is however very poorly characterized.

The overall aim of this current work then was to assess the extent to which transfer of OTC from swine manure to aquatic plants occurs and the implications of this for human exposure. Manure-water transfer was investigated by using spiked swine manure to determine desorption isotherms. Plant bioconcentration from water was quantified using three aquatic plants with contrasting morphologies and growth habits. Aquatic plants or macrophytes may be classified as floating, submerged or emergent (Zhao et al., 2012). Such plants can uptake chemicals such as tetracyclines directly from water via root cells or other immersed tissues. Watermeal (*Wolffia globosa* Hartog and Plas) is a free-floating rootless aquatic plant with no distinct stems and leaves (Rahman and Hasegawa, 2011). Cabomba (*Cabomba caroliniana* A. Gray) is a submerged plant native to South America and introduced to many Asian, Pacific and European countries where it has become an invasive weed (Wilson et al., 2007). Water spinach (*Ipomoea aquatica* Forsk.), is regarded as an emergent aquatic plant (Ko et al., 2011). It is a plant rooting at the nodes with hollow stems, 2–3 m or longer, that can float. Water spinach and watermeal in particular are widely consumed by Asian people (Zarcinas et al., 2004; Marcussen et al., 2008). Importantly in the context of this current work, both are often grown in polluted rivers and canals in Southeast Asia before harvesting (Söderström and Bergqvist, 2003). Exposure to OTC desorbed from swine manure could occur under these conditions. By combining results from swine manure desorption and plant bioconcentration, the implications for exposure of humans to OTC via this route can be assessed by comparing derived daily intake rates to the ADI.

A secondary aim with regard to bioconcentration was to investigate the extent of any translocation with water spinach since it is the upper part of the stem with foliage that is actually consumed (Marcussen et al., 2009). Translocation may be defined as the movement of a chemical from one plant part to another. It is not appropriate to refer to translocation with watermeal and cabomba. In the former, there is no differentiation into separated plant parts. In the later, the whole plant is submerged and has the same exposure to a dissolved chemical in a homogeneous aquatic system.

2. Materials and methods

2.1. Reagents

OTC (as the hydrochloride (OTC.HCl)) (>98.5% purity) for use as a standard for HPLC analysis was purchased from Dr. Ehrenstorfer GmbH, Augsburg, Germany. Commercial grade OTC.HCl used in experiments was obtained from Nova Medicine, Bangkok, Thailand and checked for impurities prior to use. Details of all other reagents used are found in Boonsaner and Hawker (2013).

2.2. Analytical protocols for manure, water and plant samples

The concentrations of OTC in manure and plant samples (sample mass approximately 10 g) were determined by adding 25 mL of McIlvaine buffer-EDTA solution to the sample and following the method described in Boonsaner and Hawker (2010). Briefly, samples were blended with an Ultra-Turrax homogenizer, then placed in an ultrasonic bath for 2 min and finally filtered through a glass fiber filter. The filtrate was passed through a conditioned SPE cartridge and OTC eluted with 2 mL of methanol. Resulting solutions were analyzed by HPLC (Waters 600, Milford, MA, USA) with Photodiode Array Detection. Analytical details are found in Boonsaner and Hawker (2013).

Water samples (100 mL) were passed through a conditioned SPE cartridge, eluted with 2 mL of methanol and analyzed by HPLC using the same conditions as for manure and plant sample extracts. Method detection limits for the analysis of OTC in plants and manure were 0.45 and 0.7 mg kg^{-1} dry weight (dw) respectively and 0.5 mg L^{-1} for water. The mean recoveries from water, plant and swine manure samples were 97%, 70% and 65% respectively. All concentrations reported herein account for these recoveries.

Analyses of selected metallic elements in swine manure were carried out by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). An Agilent 710 instrument was used, with a radio frequency generator power setting of 1.0 kW. Plasma gas (Argon) and auxiliary gas flow rates were 15 and 1.5 L min^{-1} respectively. Yttrium (371.029 nm) was used as the internal standard and analytical emission lines were 259.940 nm (Fe), 285.213 nm (Mg), 396.152 nm (Al) and 422.673 nm (Ca). The organic carbon content of manure samples was determined by the Walkley–Black wet combustion method (Tan, 1996).

2.3. Desorption of OTC from swine manure

Swine manure free from antibiotic contamination was obtained from a local farm in Nakhon Pathom province, central Thailand. The manure was sun-dried for 2 days and sieved to 2 mm size. Prior to use, the manure was autoclaved twice at 121 °C and 1.03×10^5 Pa for 15 min to minimize any microbial activity that may degrade the OTC during the timeframe of the experiments (Ratasuk et al., 2012).

OTC was spiked into swine manure by adding an OTC solution in acetone to 10 g of manure to make nominal initial concentrations of 50, 100 and 200 mg OTC kg^{-1} of swine manure. After acetone was removed, actual initial concentrations were determined to be 43, 83 and 203 mg OTC kg^{-1} (dw) of swine manure. Then, 500 mL of sterile water was added to glass jars containing contaminated swine manure to make a total solids content of 20000 mg L^{-1} or 2% (w/v). Fulhage and Pfost (2001) note that swine lagoon effluent can have a solids content up to 2% (w/v).

Nine glass jars for each OTC concentration in swine manure were prepared. All glass jars were covered in aluminum foil and placed in a dark room (28 ± 2 °C). On days 0, 1, 3, 5, 7, 9, 11, 13 and 15, one jar of each test concentration was taken and the water filtered. Duplicate 100 mL samples of filtered water from each test concentration were then analyzed for OTC by SPE and HPLC as described above. After Loke et al. (2002), sorbed OTC amounts at these times were calculated by difference between the initial amount in the system and amounts in the water. To establish the extent of OTC degradation under these conditions, a parallel control experiment was conducted comprising an aqueous OTC solution (40 mg L^{-1}) in the absence of swine manure.

2.4. Bioconcentration of OTC by aquatic plants

Watermeal, cabomba and water spinach were purchased from a local market in Nakhon Pathom province, central Thailand. Before commencing the experiments, they were analyzed by the methods described above and determined to be OTC free.