Agricultural Water Management 206 (2018) 42–47

Contents lists available at ScienceDirect

Agricultural Water Management

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

Absorption of carbamazepine and diclofenac in hydroponically cultivated lettuces and human health risk assessment

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ARTICLE INFO

Keywords:
Pharmaceuticals
Human health risk
Wastewater treatment plants (WWTPs)
Effluents
Irrigation

ABSTRACT

Due to current water shortages, the use of water from wastewater treatment plants (WWTPs) for horticultural crop irrigation is becoming increasingly common. This practice implies the risk of introducing pharmaceutical compounds into the food chain. The main aim of this work was to study the accumulation of two drugs in lettuces and their subsequent transfer into the food chain. The study focused on two widely used drugs, the anticonvulsant carbamazepine (CBZ) and the anti-inflammatory diclofenac (DCF), with different physico-chemical properties in terms of their hydrophobicity and solubility in water. Three varieties of lettuce were selected and irrigated with water containing a mixture of the two pharmaceutical compounds at different concentrations. The results show the leaves presented the highest levels of uptake and greatest bioconcentration factors in the case of CBZ; however, in the case of DCF, by contrast, the highest uptake levels and greatest bioconcentration factors were observed in the roots. For CBZ, the C_{leaf}/C_{root} ratio was greater than 1, indicating good root-to-leaf drug translocation, whereas all C_{leaf}/C_{root} ratios were less than 1 for DCF. From the data acquired, our evaluation suggests that the concentrations of CBZ and DCF detected in the edible part of the lettuces do not imply any risk to human health.

1. Introduction

The world's freshwater resources are limited and represent only a small fraction of the total water reserves (Oki and Kanae, 2006). Seventy percent of the world's freshwater is used for crop irrigation, 20% in industry and 10% is allocated to domestic use (Zimmerman et al., 2008). Pressure on water resources will continue to grow due to an ever-increasing population and climate change (Gerland et al., 2014).

Responsible techniques must be implemented to avoid water contamination. For example, those used in the management of cow manure (Hanifzadeh et al., 2017) for direct application without any treatment where most nutrients in the manure are normally washed into rivers and lakes where they cause eutrophication and potentially jeopardize the quality of drinking water. Wastewater treatment eliminates the environmental and social problems derived from wastewater release into water resources. Nowadays, we can use wastewater treatment processes to satisfy the growing demand that cities, agriculture and industry place on our water supplies in arid and semi-arid zones (Elgallal et al., 2016). Reclaimed water can be used in large-scale biomass and lipid production from microalgae (Hanifzadeh et al., 2018) and this process represents a valuable resource in meeting water demands as it helps relieve shortages and pressure on water supplies. Most irrigation with wastewater takes place in peri-urban belts around cities where there is an easily accessible supply of WWTP effluent (Hamilton et al., 2007). The agricultural use of reclaimed water is common practice in Mediterranean countries (particularly Greece, Italy and Spain) and it is increasingly used to irrigate crops intended for human consumption (Gatta et al., 2016; Pedrero et al., 2010; Libutti et al., 2018).

However, the use of reclaimed water from WWTPs represents a dilemma because different studies have shown that WWTPs are insufficient when it comes to the elimination of pharmaceuticals (Gros et al., 2010), veterinary products and other emerging organic contaminants (Calderón-Preciado et al., 2012; Goldstein et al., 2014; Macherius et al., 2012; Malchi et al., 2014; Wu et al., 2014) from urban wastewater as some of these compounds have been detected in

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https://doi.org/10.1016/j.agwat.2018.04.018
Received 13 November 2017; Received in revised form 11 April 2018; Accepted 13 April 2018
0378-3774/

wastewater used for agricultural irrigation (Calderón-Preciado et al., 2011a; Kümmerer, 2009). This, in conjunction with the complex molecular structure of pharmaceutical products and their low maximum concentration in water, means they could be entering the environment primarily by means of these effluents.

The emerging contaminants most frequently observed in WWTP effluents are: analgesics, antibacterials, antibiotics, antidepressants, anti-inflammatories, antipyretics, beta-blockers, lipid-lowering drugs, steroids, stimulants and tranquilizers (Flyborg et al., 2010; Hörsing et al., 2011; Kasprzyk-Hordern et al., 2007; Mendez et al., 2016; Miege et al., 2008; Radjenovic et al., 2007; Rosal et al., 2010).

The pharmaceuticals in this study were selected based on their: (i) presence at the inlets of different WWTPs, 420 ng L-1 for CBZ and 250 ng L-1 for DCF, (Petrovic et al., 2005); (ii) presence in surface and groundwaters in Germany (Hirsch et al., 1999; Putschew et al., 2000; Ternes, 1998; Ternes and Hirsch, 2000), Holland (Belfroid et al., 1999), Switzerland (Soulet et al., 2002), Italy (Castiglioni et al., 2004), USA (Drewes et al., 2001; Kolpin et al., 2002), Canada (Miao et al., 2004; Ternes et al., 1999), China (Chen et al., 2011; Sui et al., 2010; Yuan et al., 2013) and Brazil (Ternes et al., 1999); (iii) potential effects on human health.

In the specific case of Spain, certain pharmaceutical compounds have been detected in the effluent from various WWTPs. Approximately 20 pharmaceutical compounds were identified at 18 sampling points along the Ebro River (Petrovic et al., 2005). Another study revealed a total of 20 pharmaceuticals in seawater and 14 in sediments collected from a Mediterranean coastal lagoon (Moreno-González et al., 2015), while CBZ and DCF were identified and quantified in the influents and effluents of 12 WWTPs in the Region of Murcia (Murcia, Spain) (Fernández-López et al., 2016). There is therefore a need to evaluate the behavior of CBZ and DCF and the potential health risks they could imply if they enter into the food chain. Drug toxicity studies have been conducted on CBZ and DCF, and their potential risk to human health has also been evaluated. Individuals exposed to carbamazepine, especially Asians bearing HLA-B1502 alleles, are prone to severe hypersensitivity reactions (Thorn et al., 2011). in pregnant women, carbamazepine is considered potentially teratogenic, especially at high doses (Tomson et al., 2011). DCF induces gastrointestinal tract toxicity with clinical symptoms of small intestinal ulcers, bleeding, perforation, and strictures (Davies et al., 2000; Wolfe et al., 1999).

The accumulation of drugs in plants has been widely documented in several studies (Calderón-Preciado et al., 2011b; Goldstein et al., 2014; Shenker et al., 2011; Wu et al., 2010; Wu et al., 2012a; Wu et al., 2014). Various studies have demonstrated that crops can uptake and bioaccumulate CBZ and DCF (Herklotz et al., 2010; Winker et al., 2010; Zhang et al., 2012). This implies humans may potentially be exposed to these pharmaceuticals by consuming crops irrigated with wastewater and further research is necessary to ensure this does not pose a risk to human health.

Given the above, the objective of this study was to evaluate CBZ and DCF uptake in three varieties of lettuce irrigated with water containing a mixture of the two compounds at different concentrations, as well as to evaluate the potential risk to human health resulting from their incorporation into the food chain.

2. Materials and methods

2.1. Chemicals analyzed

The anticonvulsant carbamazepine (C4021-1G) and the anti-inflammatory diclofenac (D6899-10G), both purchased from Sigma–Aldrich (Steinheim, Germany), were selected so that we could compare the behavior, root surface biosorption and translocation into the plant of two drugs with different physicochemical properties.

Table 1 shows the structure and physicochemical properties of the pharmaceutical products.

2.2. Experimental design

The experiment was conducted in the summer of 2016 (June-August) in a greenhouse (37°47′48.88″N, 0°57′33.63″ W) owned by the Spanish National Research Council's Segura Center of Soil Science and Applied Biology (CEBAS-CSIC). The greenhouse was located in facilities adjacent to the Roldán, Lo Ferro and Balsicas WWTP (Murcia, Spain), at natural light and at temperatures ranging from 25 to 41 °C, according to data recorded by CEBAS-CSIC's weather station.

The water used in the experiment was the effluent from the Roldán − Lo Ferro and Balsicas WWTP, which receives wastewater from the communities of Lo Ferro, Roldán and Balsicas (Murcia, Spain). The reclaimed water is used for agricultural purposes. The WWTP features a primary physicochemical treatment, a secondary system consisting of an activated sludge bioreactor, a tertiary treatment based on UV irradiation, and it has a design capacity of 5500 m^3 day^{-1}. Water from the WWTP was analyzed (Appendix A Table S1) and the results at the time of the study did not reveal the presence of CBZ and DCF as their concentrations were below the limits of detection (Appendix A Table S2).

The greenhouse's surface, having an area of 680 m^2 was divided into six plots (Fig. 1) in which three varieties of lettuce were planted: Iceberg var. Gitana (IL); Mini Romaine var. Jabera (MRL); and Oak Leaf var. Kiprien (OLL). The lettuces were planted on a coconut fiber substrate (Appendix A Table S3) in bags measuring 100 × 20 × 15 cm. The plants were placed 0.25 m apart and watered by drip irrigation with four drippers per bag each operating at a flow rate of 3 L h^{-1}.

To study the degree of drugs absorption by the three varieties of lettuce, the plants were irrigated with a nutrient solution (Appendix A Table S4) dispersed in WWPT effluent containing different concentrations of CBZ and DCF.

The six greenhouse plots were divided into five experimental subplots according to the drug concentration in the water used to irrigate the three lettuce varieties. One subplot was a blank (drug-free irrigation water), while the other four were irrigated at different drug concentrations: 30, 60, 120 and 210 μg L^{-1} (each subplot contained eight plants of each lettuce variety). We prepared three replicates of each experimental subplot distributed randomly in an attempt to prevent and mitigate potential effects of the spatial situation inside the greenhouse.

An irrigation regime of 8 Liters per unit per day (1 Liter per plant per day) was applied. The mixtures of the two drugs at different concentrations were prepared in black glass jars to protect them from sunlight and prevent their degradation. Reclaimed wastewater was collected from the WWTP twice weekly to minimize any changes occurring while the water was stored in a tank near the greenhouse (Shenker et al., 2011).

All containers used to prepare the drug mixtures were washed thoroughly with methanol and acetone before use in order to eliminate any residues that could affect the study results. The plants were irrigated with the drug mixtures for a period of 13 days starting from six weeks after sowing. The experiment lasted a total of eight weeks.

2.3. Extraction and UPLC analysis

At the end of the experiment, plants from each variety were randomly selected and harvested from the middle row (to avoid border effects) and roots and leaves were analyzed. The roots were rinsed with deionized water to remove any remaining coconut fiber substrate. The roots and leaves were cut into small pieces, freeze dried and stored at −20 °C until analysis. Samples were ground to a fine powder with a porcelain mortar, according to described method of Wu et al. (2013).

Vegetable tissue was extracted as follows (Wu et al., 2012b): 0.2 g of lettuce plant sample was placed in a 50 mL glass centrifuge tube, spiked with deuterated pharmaceuticals as recovery surrogates, extracted with 20 mL methyl *tert*-butyl ether in an ultrasonic bath (50/60 Hz) for 20 min and centrifuged at 3000 rpm for 20 min. The supernatant was decanted into a 40 mL glass vial and the residue extracted using 20 mL