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Brief Research Paper

The fate of contaminants of emerging concern in wastewater-irrigated cherry trees: a preliminary assessment

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Abstract: Climate change is limiting the availability of conventional irrigation water sources. The adoption of wastewater reuse could be a valid alternative to face irrigation water shortages. Unfortunately, current wastewater purification plants are often not equipped to eliminate contaminants of emerging concern (CECs). Consequently, treated wastewater used for crop irrigation could pollute soils and plants with unknown effects of accumulation, biodegradation, and ab-/adsorption. The present study evaluates, on potted cherry trees irrigated with municipal treated wastewater, the potential accumulation/translocation of CECs in soil and plant matrices. From 17 CECs found in the wastewater, only a few were found at a concentration above 2 µg/L (*i.e.,* Candesartan and Hydrochlorothiazide). Furthermore, only Candesartan and Hydrochlorothiazide were detected in the soil medium, while no CECs were found in plant tissues (leaf). Further studies are necessary to better understand the different CECs behavior and pathways in the water-soil-plant continuum. This should go together with an implementation of analytical methods for the detection of CECs and related metabolites.

Keywords: pharmaceuticals, Candesartan, hydrochlorothiazide, municipal treated wastewater, fruit tree, irrigation.

1. Introduction

The effect of the climatic changes together with the rising population water consumption pressures the availability of freshwater resources (Thomas Riede et al., 2021). Consequently the agricultural sector, that accounts approximately for 70% of the worldwide freshwater withdrawals, needs to develop more sustainable measurements of water consumption and water reuse (Food and Agriculture Organization of the United Nations, 2017).

One promising approach to respond to water shortages is to increase the reuse of treated wastewater (TWW). TWW is usually defined as wastewater that has been treated in wastewater treatment plants (WWTPs), with different physical (primary treatment), biological (secondary treatment) and chemical (tertiary treatment) processes aimed at the removal of contaminants such as biodegradable organics, suspended solids, chemical pollutants and pathogens, for a safe discharge into the environment or for its reuse in non-potable applications (*e.g.*, irrigation) (Mancuso et al., 2021).

Nonetheless, the utilization of municipal treated wastewater (domestic wastewater or the mixture of domestic and industrial wastewater) comes with several risks and benefits for human, animal and environmental health. Positive impacts can be observed for agricultural irrigation in terms of a continuous water supply (especially during irrigation water consumption peaks), savings in macro and micronutrients for crops nutrition and reduction of river eutrophication risks (Huang et al., 2017; Zhang and Shen, 2019; Perulli et al., 2022). On the other hand, this practice also poses several risks related to pathogens (bacteria and viruses) and chemical (heavy metals, phytotoxic elements) environment contamination (Odone et al., 2024). One of the main current emerging concerns, when using wastewater sources for irrigation, is the potential contamination with pharmaceuticals, personal care products, pesticides, and industrial compounds that could be introduced into the environment and in the food chain (Tremblay et al., 2011; Das et al., 2017). These pharmaceutical and other chemical compounds could be subsumed under the term "contaminants of emerging concern" (CECs) (U.S. EPA, 2002). Their effects on ecological and human health, particularly their potential toxicology, are not foreseeable. Moreover, many CECs are not or cannot be tested or removed in municipal purification water systems (Tremblay et al., 2011) as conventional wastewater treatment plants are not designed to remove them. This leads to CECs potential accumulation in soil and crops irrigated with wastewater, posing health risks to humans or animals through the transmission or consumption of contaminated crops (Ben Mordechay et al., 2021). The list of human pharmaceutical residues commonly found in wastewater according to literature are antibiotics, anti-inflammatory drugs, anti-epileptic drugs, antidiabetic drugs, antimycotics, beta blocker, analgesic drugs, hormones and endocrine-disrupting compounds (Tremblay et al., 2011; Luo et al., 2014).

CECs introduced to the soil via irrigation, can accumulate through sorption processes and become bioavailable for plant uptake. The CECs accumulation, durability and mobilization in the soil depend on the soil composition on one side, and on the molecule's physico-chemical properties on the other. Once potentially available to the plant, the predominant entry pathway for CECs is through the root system (Zhang, 2009) where they can accumulate but also be translocated to the upper tissues driven by the transpiration flow (Fu et al., 2019). Based on several studies, the uptake and translocation of CECs in crop plant tissues has been ranked by Christou et al. (2019), from high to low occurrence, in the following order: leafy vegetables > root vegetables > cereals and fodder crops > fruit vegetables; their concentrations range from µg to mg per kg. Similar as in the soil environment, CECs plant uptake depends on the physico-chemical properties of the compounds, (*e.g.,* lipophilicity, hydrophobicity) (Christou et al., 2019; Fu et al., 2019) but also by the simple CECs concentration in the wastewater (Ben Mordechay et al., 2021).

Current research on CECs is mainly focused on plant uptake mechanisms, translocation and accumulation in plant organs of vegetable crops. Current research in perennial fruit tree (*e.g.,* avocado, banana, citrus and olive tree) is limited to a few studies (*e.g.,* Ben Mordechay et al., 2021; Mininni et al., 2023).

Based on the limited availability of research focusing on CECs behavior on fruit trees, the objective of the present study is to examine CECs in the soil-plant continuum of wastewater-irrigated cherry trees.

2. Materials and Methods

2.1. Experimental set-up

The trial was carried out in 2023 in Forchheim (Germany), in the test facility of the fruit information center OIZ (Obstinformationszentrum Fränkische Schweiz - Landratsamt Forchheim), on two-years-old, not-bearing, cherry trees (*Prunus avium* L.) ´Hudson´ cultivar, grafted on 'F 12/1' vigorous rootstock. Trees were planted in 50-L pots manually filled with a soil-substrate mixture made of 30 L substrate and 5 L sand per unit (6:1 ratio).

The applied substrate contains 70% peat bog (white and black peat) whereas the remaining 30% contains 0.5-0-8% lime, 2% sand, fertilizers, and micronutrients (https://www.plantaflor.de/en/products/home-garden/details/plantaflor-planting-soil). The trial was conducted in an open foil tunnel greenhouse with a climate-controlled ventilation system.

A total of twenty cherry trees were split into two treatments (10 tree treatment^{-1}) based on the irrigation source: i) tap water (CTRL) and ii) treated municipal wastewater (TMWW). The TMWW was subjected to the German decree of the Bavarian water legislation (https://www.gesetze-bayern.de/Content/Document/BayWG) and was locally obtained by the municipal wastewater treatment plant of Egloffstein (DMS: N 49° 43' 10.727''; E 11° 15' 6.585''). The TMWW was supplied by the municipal sewage plant after the third purification stage and transported to the experimental facility in two lightproof bulk containers of 1 m^3 volume each. Throughout the whole trial, from July 24th until

October 3rd 2023, the containers were stored at a temperature of 4 °C in a cooling chamber.

In both treatments, trees were manually irrigated and the irrigation regime varied from one to three manual water applications per week during the irrigation season (July - October), depending on the environmental conditions (data not provided) and for avoiding water leaching. CTRL and TMWW treatments received the same irrigation volume (58 L $tree^{-1}$) throughout the season (70 days).

2.2. Sample collection and CEC analyses

TMWW samples were analyzed at the beginning and at the end of the experimental trial (n=2). These samples were stored at -20 °C before being analyzed by the Jena Bios Laboratory (https://www.jenabios.de/analytik/analyseparameter/) for contaminants of emerging concern (CECs).

The wastewater samples were analyzed according to the German industrial norm DIN EN ISO 11369-F12 (1997-11). The protocol defines a method to determine selected plant treatment agents using high-performance liquid chromatography with UV detection after solid-liquid extraction (https://www.Beuth.de/de/Norm/Din-En-Iso-11369/3068569, 1997).

To analyze CECs in plant tissue and soil matrix, leaves and soil substrates were collected, in both treatments (CTRL and TMWW), at the end of the trial (October 3rd; Phenological phase: shoot growth completed and foliage still fully green).

Concerning leaves, all the trees in both treatments were defoliated. For each treatment, collected leaves were grouped in two bulk samples (made of 5 trees per each bulk) and packed in separate plastic bags. Leaf samples were stored at 4 °C and then sent, inside a Styrofoam box cooled with ice packs, to the Jena Bios laboratory.

Soil samples were taken between 5 to 30 cm soil depth to collect the middle part of the pot. One single soil bulk sample (10 soil cores per treatment) was collected for each treatment. Soil samples were stored at 4 °C and then sent, inside a Styrofoam box cooled with ice packs, to the Jena Bios laboratory.

Leaf and soil samples were analyzed according to the protocol HA JB 302, 2021-04 determining pharmaceutical products, pesticides and biocides in sludge, biowaste, and soil with HPLC-MS/MS (https://www.dakks.de/files/data/as/pdf/D-PL-19614-01-00.pdf).

Main CECs contaminant groups and their specific molecules analyzed in water, soil, and plant matrix are reported in Table 1.

Table 1. CECs specific molecules and their belonging groups analyzed in TMWW.

Contaminant groups	Specific molecules
Analgetic	Diclofenac, Fenoprofen, Ibuprofen, Antipyrin, Pentoxifylline
Antibiotics	Acetylsulfadiazin, Acetylsulfadimethoxin, Acetylsulfadimidin (-methazin), Acetylsulfamethoxazol, Acetylsulfathiazol, Acetylsulfadoxin, Acetylsulfamerazin, Amoxicillin, Ampicillin, Cefalexin, Chloramphenicol, Chlortetracyclin, Iso-chlortetracyclin, Clarithromycin, Clindamycin, Demeclocycline, Dicloxacillin, Doxycycline, Enrofloxacin, Erythromycin, Gentamicin, Imipenem, Meclocyclin, Trimethoprim, Metacyclin, Metronidazol, Oxytetracyclin, Penicillin, Ronidazole, Roxithromycin, Spectinomycin, Spiramycin, Sulfadiazin, Sulfadimethoxin, Sulfadimidin(-methazin), Sulfadoxin, Sulfamerazin, Sulfamethoxazol, Sulfathiazol, Tetracyclin, +4-Epitetracyclin, Thiamphenicol
Anti-epileptic	Carbamazepine
Antihypertensive	Candesartan, Hydrochlorothiazid
Beta-blocker	Atenolol, Metoprolol, Sotalol
Contrast media	Amidotrizoic acid, Iohexol, Iopromid, Iopamidol
Lipid-lowering drugs	Bezafibrat, Clofibric acid
Benzodiazepines	Diazepam, Medazipam

3. Results and discussion

3.1. Wastewater CECs quality

Wastewater CEC concentrations were found ranging from ng/L up to µg/L (Fig. 1). The antihypertensive drugs exhibited the highest values with 17.5 µg/L for Candesartan (CAN) and 3.45 µg/L for Hydrochlorothiazide (HCT). Other CECs detected at high concentrations (µg/L) were the analgetic agent Diclofenac (DCF) with 1.5 µg/L, the anti-epileptic drug Carbamazepine (CMZ) with 1.38 µg/L and the antiarrhythmic agent (beta blocker) Metoprolol with 1.25 µg/L (Figure 1).

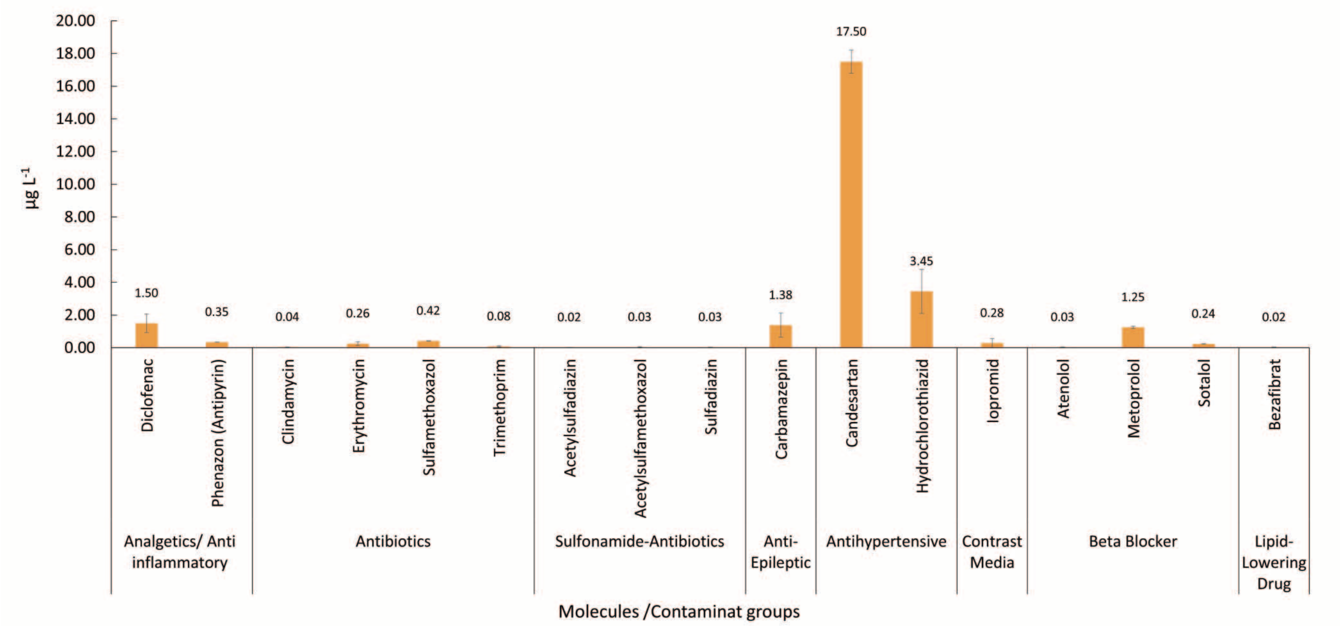

Figure 1. Concentrations of CECs found in TMWW (average ± standard deviation).

Regarding CAN, a particularly high concentration was detected in our TMWW compared to values reported by Luongo et al. (2021), who found 1712 ng/L in Bavarian WWTP effluents. Castro et al. (2019) ascertains the ubiquitous presence of the sartan group drugs in raw wastewater with an average concentration of above 1000 ng/L. Some molecules of the sartan family are known to exhibit a persistent behavior due to the high stability during chemical (oxidative) water treatments (*e.g.,* photolysis, free chlorine, ozone addition) (Blum et al., 2017; Castro et al., 2019). Nonetheless, considering the absence of hospitals or pharmaceutical industry in the residential area of the wastewater purification plant, the values found in our study are particularly high compared to the literature.

For HCT, our results are in a similar range of those of Biel-Maeso et al. (2018) who measured 2270 ng/L and 4430 ng/L mean and maximum concentration, respectively, in wastewater effluents in Spain. Similar values were also measured by Lara-Martín et al. (2014). On the contrary, Patel et al. (2019) reported HCT values between 223−233 ng/L in wastewater effluents and 223−997 ng/L for hospital effluents in Portugal.

Regarding CMZ, the values measured in our study were more than double compared to what was found by Ben Mordechay et al. (2021), that detected CMZ at 785 ng/L in wastewater for irrigation purposes in Israel. Tran et al. (2018) indicated levels of CMZ from minimum quantification values (<1 ng/L) up to 4596 ng/L in wastewater treatment plant effluents. Ternes et al. (2007) reported CMZ concentrations to be about 2000 ng/L in sewage treatment plants, stating that CMZ is not degraded nor sorbed onto sludge when passing through wastewater treatment plants and therefore suggests this compound to be used as a wastewater quality indicator.

The DCF was detected at similar concentration compared to the maximum values (1524 ng/L) found by Ben Mordechay et al. (2021). Even Tran et al. (2018) reported DCF concentrations ranging between 1−5,164 ng/L in treated effluent from different WWTPs around Europe. Ternes et al. (2007) found DCF in the wastewater effluents at 1,300 ng/L, while Christou et al. (2017) reported, for three consecutive years, mean DCF concentrations in the range between 25.80 and 49.63 ng/L.

For the beta-blocker metoprolol, our results reflect what reported by Ternes et al. (2007) who found metoprolol at 1,700 ng/L in the wastewater effluents. Tran et al. (2018) found more variable concentrations between 0−5,762 (ng/L) in treated effluents from different WWTPs in Europe. On the contrary, Ben Mordechay et al. (2021) reported only 94 ng/L in irrigation water samples.

All antibiotic substances were within 10 to 420 ng/L, with sulfamethoxazol showing the highest concentration with 420 ng/L. The overall detected CECs in our wastewater samples generally match the concentrations found in literature although the high variability registered among the studies due to the different wastewater origin and WWTPs treatment level.

3.2. Soil and leaf CEC concentrations

Concerning CAN, it was found only in TMWW (39 ng/g) while it was not detected in CTRL soil (Tab. 2). A major factor influencing CAN accumulation in the soil might be its high wastewater concentration (17500 ng/L). Indeed, when investigating water-soil-plant continuum, Ben Mordechay et al. (2022) recognized the concentration of certain CECs in the wastewater source to be the main influence on their retrieve in the irrigated soils. Nonetheless, Ben Mordechay et al. (2021) also found, for selected stimulants (caffeine, cotinine) and anticonvulsants (CMZ), a relatively low persistence in soils, even considering a high occurrence in the wastewater source. This likely explains what was found in our study where we had high wastewater CAN values (17.5 μg/L), but with only a small fraction retrieved in the soil (39 ng/g). Ben Mordechay et al. (2021) attributes this phenomenon to rapid molecules biological degradation processes. In the case of our study, it is likely that CAN was converted into a biodegradation product that we could not analyze as the break-down substances, transformation products, metabolites, and its degradation pathway were unknown to us.

Unfortunately, data comparison with other studies were not possible as current literature focuses on CAN plant uptake rather than on soil CAN concentration or accumulation.

Table 2. CECs concentrations detected in TMWW and CTRL soil samples.

CECs	TMWW (ng/g)	CTRL
Candesartan (CAN)	39.0	n.d.
Hydrochlorothiazid (HCT)	4.00	n.d.

n.d.: not detected.

Even HCT, the second highest wastewater concentrated CECs, was retrieved only in wastewater-irrigated soil samples (Tab. 2). Similar results were found by Biel-Maeso et al. (2018) who reported values ranging from 0.38 up to 1.20 ng/g in wastewater irrigated soils. Martinez-Piernaz et al. (2018) discovered significant HCT concentrations (ca. 66 ng/g) but in perlite substrate irrigated with reclaimed water for two consecutive years.

Except CAN and HCT, the two highly concentrated CECs in wastewater, all the other molecules were not retrieved in the soil samples. This could be likely explained by CECs degradation process (formation of metabolites, sub-molecules or transformation products) occurring in the soil environment, preventing its analytical detection.

Nevertheless, Ben Mordechay et al. (2021) discovered the tendency of some substances (*e.g.,* CMZ, DCF, antibiotic agents) to be persistent in soils, despite their relatively low occurrence and concentration in the irrigation water. Despite that, no CEC residues were traced in the soil analyzed in our study even considering the relatively high percentages of peat bog substrate. Soil organic matter content is known to influence positively the durability and adsorption of CECs in the soil solid phase (Ben Mordechay et al., 2021).

Regarding the plant epigeal part, no CECs were found in plant leaf tissue, even considering the high CAN and HCT concentrations in wastewater (17.5 and 3.45 µg/L). A possible explanation could be due to the characteristics of the soil used in our study (high percentages of peat bog substrate) likely able to reduce CECs plant bioavailability while facilitating CECs degradation process (Gworek et al., 2021). Furthermore, the Casparian strip could have blocked compounds entering the root cells from reaching the xylem sap flow (Ben Mordechay et al., 2022) and thus preventing the CECs translocation in upper plant parts through the transpiration flow. Our results agree with what found by Mininni et al. (2023) suggesting that most CECs are more likely to accumulate in roots and not move easily from roots to other plant parts.

4. Conclusions

Different CECs were retrieved in wastewater originated by a municipal wastewater treatment plant, but in most of the cases at very low concentrations (<2 µg/L) apart Hydrochlorothiazide and Candesartan which instead were found above 2 µg/L.

Furthermore, only two CECs were found in the wastewater-irrigated soil, Candesartan and Hydrochlorothiazide, but at low concentrations. No CECs were found in upper plant tissues (leaf). These are promising results (even considering the potted conditions) for safe wastewater reuse when irrigating fruit trees in field conditions. In any case, further understanding of CECs degradation and accumulation processes in the water-soil-plant continuum are necessary to evaluate their pathways and persistence in all matrices. Moreover, improved multi-analyte and multi-residue methods and techniques are necessary to better identify CECs and their transformation products.

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Conflicts of Interest: The authors declare no conflict of interest.

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