SHOULD WE BE CONCERNED ABOUT THE PRESENCE OF PHARMACEUTICALS DURING UNPLANNED WATER REUSE FOR CROP IRRIGATION?

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RESUMEN. El uso de agua superficial afectada por efluentes de depuradoras en agricultura puede implicar la exposición de cultivos a contaminantes de preocupación emergente. Este trabajo tiene como objetivo evaluar si los procesos de atenuación natural mitigan la concentración de 25 fármacos y productos de transformación (PTs) o si son absorbidos por el cultivo, en este caso maíz, induciendo un riesgo potencial para la salud humana. El método del umbral de preocupación toxicológica se ha utilizado para evaluar el posible riesgo para la salud humana. Para ello, se recolectaron muestras de diferentes matrices (agua de riego, de infiltración, suelo agrícola, maíz) donde se analizó el contenido en fármacos y PTs. Los resultados demuestran la atenuación de la mayoría de los contaminantes investigados. El consumo de maíz no representa ninguna amenaza para la salud humana; sin embargo, se requeriría realizar más pruebas para determinar la toxicidad del 4AAA y del analgésico acetaminofén.

ABSTRACT. The use of surface water impacted by wastewater treatment plant effluents for crop irrigation may imply the exposure of crops to contaminants of emerging concern. This research aims to evaluate under real field conditions if natural attenuation processes mitigate the concentration of 25 pharmaceuticals and transformation products (TPs) or if they are taken up by the crop (i.e. maize) inducing a potential risk to human health. The threshold of toxicological concern approach (TTC) has been used as a first screening for assessing possible risk to human health. Samples from different matrices (irrigation water, infiltrating water, agricultural soil, maize) were collected and analysed for pharmaceutical and TP content. Results demonstrate the attenuation of the majority of target contaminants. Consumption of maize does not pose any threat to human health; however additional toxicity test would be required for the metamizole TP 4AAA and the analgesic acetaminophen.

1.- Introduction

The unintentional use of surface water highly impacted by wastewater treatment plant (WWTP) effluents is defined as unplanned water reuse (Drewes et al., 2017). When these impacted waters are used for crop irrigation, there is a potential risk linked to the introduction of undesirable compounds into the food chain (de Santiago-Martín et al., 2020). Among these compounds, pharmaceuticals, a broad class of contaminants of emerging concern, are more than frequently found in surface waters receiving urban effluents (Meffe and de Bustamante et al., 2014). Similarly to other types of contaminants, pharmaceuticals undergo natural attenuation processes in soil that can mitigate their propagation to other environmental compartments or they can be taken up by the crop (Carter et al., 2019). The efficiency of the attenuation and the magnitude of crop uptake depends on several factors such as pharmaceutical specific properties, soil characteristics and the bioaccumulation potential of the crop itself (Fu et al., 2018; Cristou et al., 2019; Li et al., 2019).

Many studies available in the literature tackle natural attenuation and plant uptake processes using laboratory scale investigation under simplified conditions (Bhalsod et al., 2018; Klement et al., 2020; Kodešová et al., 2019). However, assessing contaminant natural attenuation and uptake by crop plants under actual farming conditions are essential to provide solid data about the real magnitude of the processes (Malchi et al., 2014; Christou et al., 2019).

In this perspective, the present research aims to investigate the extent of pharmaceutical attenuation processes and the capacity of maize, the cereal with the world largest production (FAOSTAT, 2018), to bioaccumulate them in subaerial and aerial vegetative tissues. To mirror the broad range of pharmaceuticals that every day are discharged into the environment, we selected a set of compounds (including transformation products - TPs) belonging to different therapeutic classes: analgesics, anti-inflammatories, antibiotics, cardiovasculars, lipid regulators, antidiabetics, antiulcer, psychiatric drugs and lifestyle compounds.

2.- Materials and methods

2.1 Study area and sampling strategy

This research has been carried out in an agricultural parcel located south of the city of Madrid (Spain), in an area where 10,000 ha are cultivated to produce mainly maize. The agricultural field is irrigated by gravity-fed surface system supplied by the Jarama river. The river is strongly impacted by the effluents of the capital largest WWTPs whose contribution can locally constitute up to 83% of the river flow (MAPAMA, 2015; CHT, 2019). During summer 2019, sampling campaigns were carried out to collect samples of irrigation water and water infiltrating through the vadose zone at 30 cm soil depth (in all irrigation events), agricultural soil (before and after the irrigation campaign) and maize differentiating among roots, stem-leaves and fruit (after the irrigation campaign, few days before harvesting).

The irrigation water (Ir) was sampled from the nearby channel that distributes water to the agricultural field. The infiltration water was collected at two different times: 1) approximately 4 hours before the shutdown of the irrigation during high hydraulic loads and, hence, rapid infiltration fluxes and 2) approximately 2 hours after the shutdown, when the lower hydraulic loads cause slower infiltration. To collect the infiltration water threes stainless steel suction cups installed at a distance between them of approximately 30 m were used. When analyses of water samples could not be performed immediately after retrieval, they were stored at -20°C.

About 2 kg of soil were collected using a manual auger in the proximity of each suction device and each sample was considered individually. Prior to analysis, samples were air-dried, gently crushed and passed through a 2-mm sieve. A representative sample for the analyses was obtained by quartering the pre-processed soil samples and stored in high density polyethylene (HDPE) bottles. A portion of the soil sample was successively frozen and lyophilized for pharmaceutical and TP analyses.

A total of 9 specimens of maize, 3 nearby each soil sampling point, were collected. In the laboratory, each plant was cut separating the roots, the stem/leave system and the fruit. The roots were rinsed first with tap water to remove the major part of the soil residues, and then with ultrapure water. Leaves and stems were washed with ultrapure water to remove dust and insects. The corn fruits were shelled and also washed with ultrapure water. Roots, leaves, stems and grains were dried in an oven at 50ºC. Plant tissue samples were frozen and lyophilized for analysis of pharmaceuticals and TPs.

2.2 Analytical methods

Liquid and solid samples were subjected to different treatments based on organic solvent extractions and pre-concentration procedures. The quantification of pharmaceuticals and TPs in the obtained extracts was carried out using a liquid chromatograph (1200 series, Agilent Technologies, USA) coupled to a triple quadrupole (QQQ) mass spectrometer (6495A, Agilent Technologies), equipped with an electrospray ionization (ESI) interface, in positive and negative mode.

2.3 Crop uptake and health risk assessment

To evaluate potential health risk for consumption of maize containing target contaminants, we applied the threshold of toxicological concern approach (TTC). Recently, the EFSA carried out a public consultation to receive input from the scientific community and interested parties on the draft prepared in 2018 about the guidance on the use of the TTC approach in food safety assessment (EFSA, 2019a). This resulted in a final updated version of the guidance in 2019 (EFSA, 2019b). EFSA corroborates that the TTC approach is a "*pragmatic screening and prioritization tool for use in food safety assessment*". The approach can be used when there are limited toxicity data (EFSA, 2019b; Evans et al., 2015; Kroes et al., 2000) as a tier 0 assessment to highlight the necessity for specific toxicity analysis when the consumption of substances, in this case pharmaceuticals, is higher than the TTC value. The approach is based on the comparison of substance molecular structure with structural alerts to assign each pharmaceutical to the corresponding category (Class I, II, III) through the Cramer classes decision tree implemented in the software Toxtree (Patlewicz et al., 2008, v. 3.1.0). The TTC values of 30.0, 9.0 and 1.5 µg kg^{-1} day^{-1} were defined for Class I, II and III, respectively (Munro et al., 1996). According to Kroes et al. (2004), a TTC value of 0.0025 µg kg^{-1} day^{-1} was used for potential genotoxic substances. Consumption of pharmaceuticals higher than the TTC value indicates a possible risk of exposure and the necessity for specific toxicity analysis along with implementation of an additional risk assessment method. On the other hand, pharmaceuticals with exposure below the TTC threshold have an unlikely probability to cause adverse effects on humans.

3.- Results and discussion

3.1. Pharmaceuticals occurrence and attenuation

The 25 pharmaceuticals and TPs have been detected in each irrigation sample with concentration ranging from 8.94 ng L^{-1} for the antidepressant citalopram to 12,867 ng L^{-1} for the antidiabetic metformin. The drugs with the highest levels of concentration (> 1,000 ng L^{-1}) were, beside the aforementioned metformin (average 5,825 ng/L), the metamizole TP 4FAA (average 2,966 ng L^{-1}) and the antihypertensive valsartan (average 1,036 ng L^{-1}). The concentration of pharmaceuticals and TPs in the irrigation (Ir) and infiltrating water (1: rapid; 2: slow – under the two different infiltration times) are represented in Figure 1.

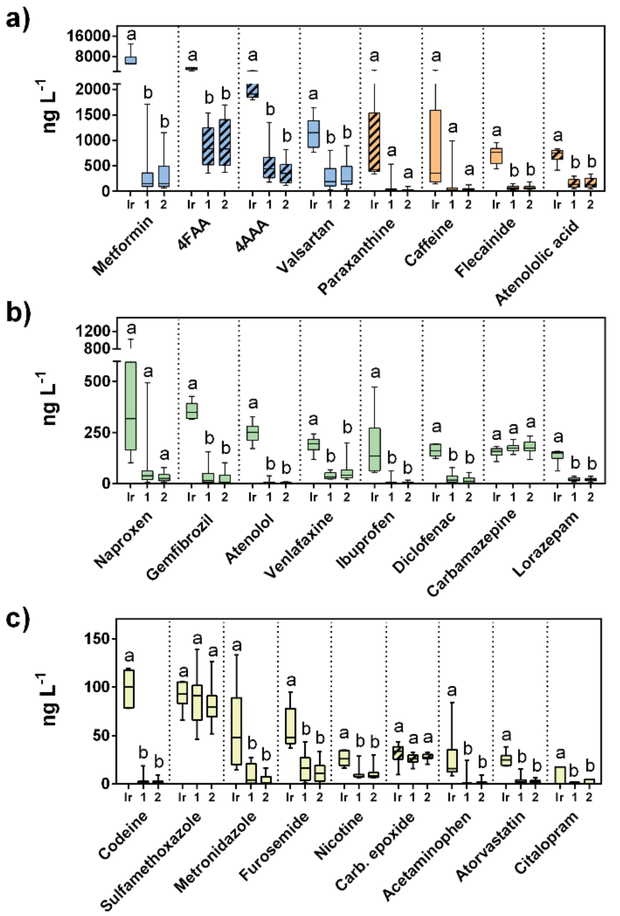

Fig. 1. Concentration of target pharmaceuticals and transformation products (TPs) in: i) irrigation water (Ir), ii) infiltrating water under high hydraulic loads (1: rapid), iii) infiltrating water under low hydraulic loads (2: slow). Substances are listed in order of decreasing concentration in the irrigation water (> 1,000 ng L^{-1} blue, 500-1,000 ng L^{-1} orange, 100-500 ng L^{-1} green, < 100 ng L^{-1} yellow). The black frame indicates TPs. For each compound, different letters indicate a statistically significant difference at $p < 0.05$ by ANOVA test.

The ANOVA test applied to concentrations data in water reveals the absence of a significant difference between samples 1 and 2. Hence, data rule out infiltration velocity in the vadose zone as a factor controlling the attenuation of selected drugs. Such a result is to some extent surprising since residence times in the vadose zone are known to affect degradation rates. The environmental and input concentration variability occurring under field conditions may obscure differences in removals between infiltration stages.

Considering both samples (1 and 2) as replicates, average removal percentages are higher than 60%, with the exception of carbamazepine, its TP carbamazepine epoxide and sulfamethoxazole that behave persistently (-28.95%, 1.36% and -0.48%, respectively). Carbamazepine and its TP carbamazepine epoxide are known to be recalcitrant to degradation. The persistence of sulfamethoxazole can be related to its negative charge under environmental conditions that may counteract sorption onto soil colloids (clay and organic matter). Proof of this is the low content measured in the soil of the agricultural parcel (1.75 ng g^{-1}).

Concerning biodegradation, sulfamethoxazole has been described to degrade under both aerobic (Martínez-Hernández et al., 2016) and anaerobic conditions (Schmidt et al., 2004). In our study, the persistence of sulfamethoxazole occurs under the anaerobic conditions promoted by the gravity-fed irrigation systems. Even when removals are high, leaching concentrations can still have some concern in view of contaminant propagation to other environmental compartments (e.g. groundwater). This is the case of the metamizole TPs 4FAA and 4AAA, whose average leaching concentrations are above 400 ng L^{-1}.

Pharmaceutical contents in the soil are in the order of ng g^{-1} and with few exceptions, there are not significant differences between the two sampling campaigns. Those pharmaceuticals with more affinity to the agricultural soil (flecainide > venlafaxine > metformin) are positively ionized at the environmental pH and their sorption onto soil can be explained by electrostatic interactions with negative charged soil surfaces such as the organic matter and clay minerals and by cation exchange processes.

3.2. Crop uptake

Data about pharmaceutical and TP contents in the different tissues of the maize indicate that, at variable extent, the plant is able to bioaccumulate many of the investigated substances, mainly in the roots (Table 1). With the exception of gemfibrozil in the fruit, none of the pharmaceuticals negatively charged under field conditions were quantified. The negative electrical potential across the plant cell membranes may be responsible for repulsion of anionic pharmaceuticals. However in the case of weak organic acids such as gemfibrozil (pKa= 4.75), the molecule may become uncharged in the rhizoplane favouring plant uptake processes (Malchi et al., 2014). On the other hand, neutral and cationic drugs show higher potential to be bioaccumulated by maize (Table 1).

Table 1. Pharmaceutical and TP contents in the different tissues of the maize. Only data from those contaminants quantified at least in one vegetative tissue are provided. In parenthesis, the ionization status under environmental pH is indicated. LOQ: Limit of quantification.

Pharmaceutical	**Roots**	**Stem-Leaves**	**Fruit**
		ng g^{-1}	
4AAA (N)	<LOQ	ND	0.15 ± 0.04
Paraxanthine (N)	0.80 ± 0.13	<LOQ	0.12 ± 0.02
Caffeine (N)	8.42 ± 1.72	6.58 ± 1.79	1.17 ± 0.16
Flecainide (+)	18.0 ± 0.63	ND	<LOQ
Atenololic Acid (+/-)	<LOQ	ND	2.03 ± 0.63
Gemfibrozil (-)	ND	ND	0.36 ± 0.08
Atenolol (+)	<LOQ	ND	0.05 ± 0.01
Venlafaxine (+)	4.22 ± 0.18	ND	<LOQ
Carbamazepine (N)	3.57 ± 0.21	ND	<LOQ
Codeine (+)	ND	ND	0.02 ± 0.01
Nicotine (+)	9.79 ± 0.71	2.24 ± 0.33	2.24 ± 0.27
Carbamazepine epoxide (N)	0.38 ± 0.03	ND	<LOQ
Acetaminophen (N)	11.7 ± 1.27	6.44 ± 1.64	<LOQ

For neutral pharmaceuticals, hydrophobicity (expressed as $\log K_{ow}$) is often described as the factor that most influences the process of the uptake. The lipidic content of the roots is an additional parameter controlling the fate of neutral and/or hydrophobic chemicals (Malchi et al., 2014). Among neutral pharmaceuticals, the highest accumulation in roots is observed for acetaminophen (11.7 ± 1.27 ng g^{-1}) and caffeine (8.42 ± 1.72 ng g^{-1}). Cationic pharmaceuticals, such as nicotine, codeine and atenolol can be translocated by organic cation transporters (OCTs) and/or through non-selective cation channels (Fu et al., 2019). Cation transporters have been described in maize (Yang et al., 2020) indicating that bioaccumulation of these drugs is likely to occur through this mechanism.

3.3 Health risk assessment

Pharmaceuticals detected in the maize fruit are all assigned to Class III, a category that includes substances for which strong initial presumption of safety is not allowed or whose structural properties suggest significant toxicity. The only exception is venlafaxine that belongs to Class II (intermediate order of oral toxicity) (Patlewicz et al., 2008). Results indicate an insignificant threat to human health since calculated daily consumption are far above the European daily intake (DI) of maize (26.86 g d^{-1}). However, values closer to the DI have been calculated for the metamizole TP 4AAA and the nervous system acting drug acetaminophen. For these pharmaceuticals, a consumption of 103.00 and 26.50 g d^{-1}, respectively, are enough to reach the TTC in toddlers. Both pharmaceuticals are recognized as having potential genotoxicity and their TTC is only 0.0025 µg kg^{-1} d^{-1}. Results indicate that additional toxicity tests are recommended for these two compounds.

4.- Conclusions

This research demonstrates that unplanned water reuse for agricultural practices should be carefully evaluated as a route of exposure to pharmaceuticals and TPs. All investigated drugs have been detected in surface water and some of them appeared with concentrations in the order of µg L^{-1}. Natural attenuation processes occurring in the first layer of the unsaturated zone are very effective to abate concentrations of most pharmaceuticals and TPs. However for substances occurring with high concentrations in the irrigation water, leaching levels are higher than those observed for pharmaceuticals behaving persistently. Sorption onto soil is a mechanism able to buffer infiltration of cationic pharmaceuticals mainly. At variable extent, maize is able to bioaccumulate many of the investigated drugs. Concentrations in the roots are in general higher than concentration in the fruit indicating that pharmaceuticals are not readily translocated through the plant. Results also confirm the theory about plant uptake processes as preferentially occurring for cationic and neutral substances. According to the TTCs, the consumption of the maize would not pose any threat to human health in terms of pharmaceutical intake. However, additional toxicity tests would be required only for the metamizole TP 4AAA and the nervous system acting drug acetaminophen.

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