Micropollutant uptake in tomato fruit after fertigation with reclaimed wastewater in Mediterranean water-scarce area

Sofia Semitsoglou Tsiapou[1], Monica Brienza[2], Vincenzo Trotta[2], Samia Khadhar[3], Nouha Khiari[3], Lúcia Santos[1], Sara Rodríguez-Mozaz[1], and Gianluigi Buttiglieri[1]

[1] *Institut Català de Recerca de l'Aigua (ICRA-CERCA) – 17002, Girona, Spain*

[2] *Università degli Studi della Basilicata (UNIBAS) – Via Nazario Sauro 85, 85100, Potenza, Italy*

[3] *Centre de Recherche et Technologies des Eaux (CERTE) – Technopole Borj-Cedri, Soliman 8020, Tunisia*

ABSTRACT

Introduction

By 2050, water scarcity is estimated to affect more than half of the world's population (The United Nations WWDR, 2018). In the Mediterranean region, particularly in dry zones, increased tourist activity and wasted water resources pose significant challenges, therefore unconventional practices that lead towards a circular economy are sometimes unavoidable. One such viable solution, for both water and valuable nutrients reclamation, is the reuse of treated wastewater for crop irrigation. Even though both water and valuable nutrients are reclaimed with this practice, emerging organic contaminants of various sources are not usually fully removed in wastewater treatment plants, posing the challenge of contaminant uptake by the root/leaf/fruit system of the irrigated crops (Calderon-Preciado et al., 2011).

The focus of this work was to study the micropollutant uptake from tomato plants cultivated using reclaimed water as irrigation source under various conditions and in different Mediterranean countries; Italy (greenhouse), Tunisia (field site), Morocco (field site) and Greece (hydroponic greenhouse). The tomato plant was chosen as a representative crop, since it is the most important vegetable crop cultivated in the Mediterranean region, with the bobcat tomato variety selected as the common denominator for all countries. This variety was selected via experiments conducted at greenhouse scale at UNIBAS (Italy), based on factors such as commercial availability of seeds, market availability, resistance to pathogens and salinity. The presence *of Trichoderma harzianum* as bio-stimulant symbiont organism on the contaminant uptake was also investigated.

More specifically, the focus points were to: (i) study the uptake mechanism of micropollutants into the soil-plant system; (ii) estimate the role of a bio-stimulant symbiont organism on that uptake; and (iii) evaluate crop yield and soil impact at field scale. In this context, the analytical approach was first applied to estimate the micropollutant uptake in tomato samples with/without *Trichoderma harzianum* irrigated in greenhouse conditions with municipal secondary wastewater effluent. The same analytical approach was applied to the first batch of tomatoes from the field site in Tunisia, where soil irrigated by wastewater effluent in the last 40 years was treated with either municipal wastewater effluent or groundwater, and control experiments involved groundwater irrigation on soil never exposed to any wastewater effluent. Parameters monitored were soil characterization before/after the season, soil salinity, moisture, physicochemical characterization of the irrigation water, as well as micropollutant analysis in soil, water and tomato fruit. Relevant crop yield difference was observed between tomatoes irrigated with wastewater and with groundwater. Tomatoes subjected to all the abovementioned

conditions are currently being analyzed for the selected micropollutants, and in the case of Tunisia and Morocco, tomatoes harvested from two different seasons will be analyzed.

Methodology

Fourteen emerging contaminants (antibiotics: clindamycin, sulfamethoxazole, N-acetyl-sulfamethoxazole, trimethoprim, clarithromycin; psychiatric drugs: venlafaxine, N-desmethyl-venlafaxine, O-desmethyl-venlafaxine; β-blockers: metoprolol, metoprolol acid; pesticides: fipronil, fipronil-sulfide, fipronil-sulfone, fipronil-desulfinyl) were selected based on occurrence in the selected countries, persistence, toxicity, predicted bioaccumulation data (Castaño-Trias et al., 2024) and the EU watchlist 2022/1307.

Immediately after harvest, the tomatoes were freeze-dried, milled and shipped to ICRA for analysis. Sample preparation involved extraction by QuEChERS and dSPE PSA-C18 clean-up (adapted protocol, Montemurro et al., 2020). In short, 1 g of freeze-dried and milled crop leaves was placed in a 50 mL falcon tube and hydrated with 9mL HPLC water. The tubes were vortexed, shaken for 2 min at 2500 rpm and left to hydrate for 1 h. 10 mL of acetonitrile and 50 µL of formic acid were added in the tubes, vortexing was repeated and the extraction salts (1 g NaCl and 4 g MgSO4) were added in the tubes. The mixture was instantly shaken to prevent crystalline agglomerate formation. Tubes were shaken as before and centrifuged at 4°C for 10 min at 4000 rpm. The supernatant, containing the organic phase, was transferred into glass tubes, and left overnight at -20°C for the precipitation of fatty acids and waxes. Right before analysis, the clean-up step involved the transfer of 6 mL of the supernatant into the PSA (primary secondary amine) tubes (150 mg PSA, 150 mg C18, 900 mg MgSO4) and the mixture was centrifuged at 4°C for 5 min. For all samples, 1 mL of the supernatant was spiked with the internal standard mixture at a concentration of 20 µg/L, the samples were evaporated until dryness under nitrogen at room temperature, reconstituted with 1 mL of water/20% methanol solution and injected for UHPLC-MS/MS analysis. Fipronil and its metabolites were analyzed in Negative Ionization (NI) mode, and the rest of the micropollutants in Positive Ionization (PI) mode (Castaño-Trias et al., 2023).

Results

The results so far include the tomatoes from the Italian greenhouse (varieties bobcat and nano) and the tomato samples from Tunisia (bobcat variety) of the first season (both harvested in summer 2023). Matrix-match calibration curves were prepared and good linearity was shown for all of them ($R^2 > 0.998$) The QuEChERS-UHPLC-MS/MS methodology yielded high recovery rates for all selected micropollutants (76-100%).

The presence of the bio-stimulant symbiont *Trichoderma harzianum* on the contaminant uptake by bobcat and nano tomato was investigated in greenhouse conditions in Italy with either tap water (i.e., control), municipal secondary wastewater effluent (WW), or secondary wastewater followed by chlorination (WW+Cl). Three antibiotics – sulfamethoxazole, clarithromycin and venlafaxine– were found in bobcat tomato cultivated with Trichoderma (range of 18-351 ng/g d.w.); the first two were quantified with WW irrigation and all three with WW+Cl irrigation. In the case of tomato cultivated without Trichoderma, sulfamethoxazole and clarithromycin were still detected in both cases, but in lower concentrations (62-106 ng/g d.w.).

Venlafaxine and clarithromycin were the only two micropollutants detected in the nano tomato, with clarithromycin present with WW irrigation in both the presence and absence of Trichoderma (similar levels of around 50 ng/g d.w.) as well as with WW+Cl irrigation, and venlafaxine at double the concentration of clarithromycin when WW irrigation was used in the presence of Trichoderma.

Overall, the presence of Trichoderma seemed to promote higher uptake of micropollutants via the roots and into the upper parts of the plant. This finding was expected, since among biofertilizers, fungi from the genus Trichoderma can improve protection against phytopathogens and increase the plant's tolerance to phytophagous insects. Also, fungal mycelium secretes compounds that promote root branching, thus improving uptake of nutrient, water and possibly contaminants.

Analysis of the target micropollutants was also carried out in bobcat tomato from a field site in Tunisia, where soil irrigated by wastewater effluent in the last 40 years was treated with either municipal wastewater effluent or groundwater, and irrigation with groundwater on soil never exposed to wastewater served as control. None of the studied micropollutants was detected in the tomato samples from Tunisia, which could be explained by the sandier nature of the field soil in the area, which would not retain micropollutants as easily as another type of soil.

Obviously, the use of these compounds regarding their properties as pharmaceuticals, antibiotics and pesticides in each country, which will be reflected in the analysis of the irrigation water used, as well as their retention in the roots of the plants, to be reflected in the soil analysis for each case, will be combined with the crop data, to facilitate a better understanding of the behavior of these compounds in the soil-water-crop system.

A second sampling batch from both Tunisia and Morocco, and a summer sampling from Greece, are currently being analyzed for the target micropollutants. Eventually, the data from this study will serve for the assessment of potential risk to human health related to tomato consumption fertigated with reclaimed wastewater.

REFERENCE

Calderon-Preciado, D., Jimenez-Cartagena, C., Matamoros, V., Bayona, J.M. (2011). Screening of 47 organic microcontaminants in agricultural irrigation waters and their soil loading. Water Research, 45, 221-231

Castaño-Trias, M., Rodriguez-Mozaz S., Buttiglieri, G. (2023). A decade of water monitoring in a Mediterranean region: pharmaceutical prioritisation for an upgraded analytical methodology. Journal of Environmental Nanotechnology, Monitoring & Management, 20, 100850

Castaño-Trias, M., Rodriguez-Mozaz, S., Verlicchi, P., Buttiglieri, G. (2024). Selection of pharmaceuticals of concern in reclaimed water for crop irrigation in the Mediterranean area. Journal of Hazardous Materials, 466, 133538

Montemurro, N., Orfanioti, A., Manasfi, R., Thomaidis, N. S., & Pérez, S. (2020). Comparison of high resolution mrm and sequential window acquisition of all theoretical fragment-ion acquisition modes for the quantitation of 48 wastewater-borne pollutants in lettuce. Journal of Chromatography A, 1631. https://doi.org/10.1016/j.chroma.2020.461566

World Water Assessment Programme (Nations Unies), The United Nations World Water Development Report 2018 (2018). United Nations Educational, Scientific and Cultural Organization, New York, United States (www.unwater.org/publications/world-water-development-report-2018/)

ACKNOWLEDGMENTS

Authors acknowledge funding from Project PCI2022–132980 (SAFE) funded by MCIN/AEI/10.13039/501100011033 and the European Union Next Generation EU/PRTR. The Ultra-Performance Liquid Chromatography Triple Quadrupole Mass spectrometry (UPLC-MS) hybrid Linear Ion Trap (LIT), Acquity UPLC-MS QTRAP 5500, Waters-SCIEX facility received support from the CERCA Institute through the CERCAGINYS program, funded by the Spanish Ministry of Science and Innovation. The authors are grateful for the support from the Economy and Knowledge Department of the Catalan Government through a Consolidated Research Group (ICRA-TECH – 2021 SGR 01283 and ICRA-ENV - 2021 SGR 01282).