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Uptake of different pharmaceuticals in soil and mycorrhizal artichokes from wastewater

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Abstract:	The irrigation with treated wastewater is among the main anthropogenic sources for the release of pharmaceuticals (PhACs) into the soils and their translocation into crops, with possible toxic and adverse effects on humans. Thearbuscular mycorrhizal fungi (AMF) can be employed for the reduction of organic soil pollutants, even if their efficiency depends on the mycorrhizal fungi, the plant colonized, and the type and concentration of the contaminant. This study aimed to evaluate the uptake of PhACs from wastewaters of different quality used for the irrigation of mycorrhizal artichoke plants, the presence in their edible parts and the role of the arbuscular mycorrhizal fungi. The research was carried out on artichoke plants not inoculated and inoculated with two different AMF and irrigated with treated wastewater (WW), fresh water (FW) or enriched fresh water (EW). The inocula were a crude inoculum of Septoglomus viscosum (MSE) and a commercial inoculum of Glomus intraradices and Glomus mosseae (MSY). The results of the present study showed that carbamazepine and fluconazole were found in the artichoke only with EW irrigation. The mycorrhizal plants showed a reduction of the

	pharmaceutical's uptake, and within the AMF, MSE was more effective in preventing their absorption and translocation.
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Environmental Science and Pollution Research

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On behalf of all Authors, I like to submit the manuscript entitled "Uptake of different pharmaceuticals in soil and mycorrhizal artichokes from wastewater" by Francesco De Mastro, Gennaro Brunetti, Giuseppe De Mastro, Claudia Ruta, Donato Stea, Sapia Murgolo, Cristina De Ceglie, Giuseppe Mascolo, Filomena Sannino, Claudio Cocozza and Andreina Traversa.

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I look forward to hearing from you about this matter at your nearest convenience.

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Uptake of different pharmaceuticals in soil and mycorrhizal artichokes from wastewater

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Abstract

The irrigation with treated wastewater is among the main anthropogenic sources for the release of pharmaceuticals (PhACs) into the soils and their translocation into crops, with possible toxic and adverse effects on humans. The arbuscular mycorrhizal fungi (AMF) can be employed for the reduction of organic soil pollutants, even if their efficiency depends on the mycorrhizal fungi, the plant colonized, and the type and concentration of the contaminant.

This study aimed to evaluate the uptake of PhACs from wastewaters of different quality used for the irrigation of mycorrhizal artichoke plants, the presence in their edible parts and the role of the arbuscular mycorrhizal fungi. The research was carried out on artichoke plants not inoculated and inoculated with two different AMF and irrigated with treated wastewater (WW), fresh water (FW) or enriched fresh water (EW).

The inocula were a crude inoculum of *Septoglomus viscosum* (MSE) and a commercial inoculum of *Glomus intraradices* and *Glomus mosseae* (MSY). The results of the present study showed that carbamazepine and fluconazole were found in the artichoke only with EW irrigation. The mycorrhizal plants showed a reduction of the pharmaceutical's uptake, and within the AMF, MSE was more effective in preventing their absorption and translocation.

Keywords: contaminants of emerging concern, plant organs, irrigation, *Septoglomus viscosum*, *Glomus intraradices*, *Glomus mosseae*

1. Introduction

The increasing demand of water for irrigation has focused the attention on the reuse of treated wastewaters in agriculture. The Mediterranean basin is characterized by a scarcity of surface and ground waters, thus treated wastewaters represent a realistic alternative to their depletion (Lavrnić et al. 2017; Lorite et al. 2018). In this perspective, it has been estimated a reuse of more than 3000 $Mm^3\ yr^{-1}$ of treated wastewaters in agriculture by 2025 in the sole Europe (Camacho-Arévalo et al. 2021). A positive aspect of reusing treated wastewaters is related to their content of plant nutrients, such as nitrogen and phosphorus (Gatta et al. 2016; Kinney et al. 2006), and dissolved effluent organic matter (Michael-Kordatou et al. 2015), that can reduce the application of inorganic fertilizers and improve the nutrient soil-plant ratio. In contrast, in most cases, contaminants of emerging concern (CECs) remain in waters after conventional wastewater treatments because of their refractoriness to microbial degradation (Camacho-Arévalo et al. 2012; Loraine and Pettigrove 2016), and they can enter the food chain and persist for a long time in the environment (Olowoyo and Mugivhisa 2019; Wang et al. 2005).

CECs are compounds largely used for several aims (personal care products, drugs, pesticides, nanomaterials, flame retardants, hormones) including pharmaceuticals (PhACs) that are widely used as analgesics, anesthetics, antimicrobial, anti-inflammatory, antibiotics in human and veterinary fields. Several studies have shown their presence in treated wastewater used in agriculture (Frascaroli et al. 2021; Gorovits et al. 2020; Semerjian et al. 2018). Plants exposed to CECs can uptake and accumulate these contaminants in their tissues from few $\mu g\ kg^{-1}$ to few $mg\ kg^{-1}$ (Christou et al. 2016; Dodgen et al. 2015; Tanoue et al. 2012; Wu et al. 2012). However, the crops uptake of CECs is difficult to predict because it is influenced, on one hand, by their physico-chemical properties and, on the other hand, by structure and chemical properties (pH, mineral concentration, cation exchange capacity, soil organic matter) of recipient soils (Miller et al. 2016; Park and Huwe 2016; Vasudevan et al. 2009; Wu et al. 2013). For example, aerated soils favour the

uptake of CECs by plants (Christou et al. 2019), while a soil pH higher than the pKa of the CECs hinders their uptake by crop plants due to repulsion forces between the negative form of contaminant and the negatively charged root epidermis (Goldstein et al. 2014; Miller et al. 2016). Plants grown in clay soils or soils rich in organic matter show a lower uptake of CECs compared to those grown in sandy soils or soils containing a low amount of organic matter (Goldstein et al. 2014; Malchi et al. 2014). The effects of CECs on plants depend on the species, plant organ, type of contaminant and its concentration (Minden et al. 2017) and they can alter the biomass production due to the reduction of root length, number of leaves, shoots length, root/shoot ratio, fresh/dry weight ratio (Bradel et al. 2000; Li et al. 2011; Liu et al. 2009; Michelini et al. 2012; Piotrowicz-Cieslak et al. 2010; Yang et al. 2010).

The arbuscular mycorrhizal fungi (AMF) positively influence the plants growth by promoting the absorption of water, phosphorous (Hart and Forsythe 2012), nitrogen (Leigh et al. 2009) and micronutrients (Kim et al. 2010; Seres et al. 2006; Toler et al. 2005), and consequently the stress tolerance (Xu et al. 2018). The AMF also improve the soil structure by binding soil particles into stable aggregates through the production of glomalin (Rillig 2004; Wright and Upadhyaya 1996, 1998). The AMF can influence the uptake of heavy metals, as reported by many authors (Brunetti et al. 2017; Chan et al. 2013; Riaz et al. 2021). In addition, the AMF can also increase the activities of soil oxidoreductases enzymes able to catalyse the degradation and the transformation of aromatic compounds (Criquet et al. 2000; Liu et al. 2004). Previous studies reported that AMF influence the transport and distribution of CECs in plants, promoting their accumulation in roots (Debiane et al. 2009; Langer et al. 2010) and protecting the aerial parts of crops. Through a modification of the composition of microbial community and root exudates (Bouwmeester et al. 2007; Gomez-Roldan et al. 2007; Hage-Ahmed et al. 2013; Steinkellner et al. 2007), the mycorrhizae affect the microbial degradation of CECs and their fate in soils and plants (Banks et al. 1999; Reilley et al. 1996).

The present study aimed to evaluate the fate of different PhACs in soil when irrigated with waters showing different quality, i.e., groundwater available on the experimental site, as is or spiked

with selected PhACs at a concentration of 200 ppb, and a treated wastewater coming from a wastewater facility. In addition, authors investigated the effects of two AMF i) in the PhACs fate in soil, and ii) in the uptake of the selected PhACs by artichoke plants when irrigated with the aforementioned waters.

2. Material and methods

2.1. AMF and plant material

Crude AM inoculum of *Septoglomus viscosum* H.T. Nicolson (syn. *Glomus viscosum*) (MSE) was multiplied as pure culture on strawberry (*Fragaria* x *Ananassa*) plants chosen as the host crop for its high mycotrophy, in accordance with the Dalpe´ and Monreals method (2004). The MSE inoculum consisted of sandy soil containing spores, external mycelium, and infected strawberry root fragments. MSE was compared to the commercial AMF Aegis (Italpollina spa, Verona, Italy) composed by *Glomus intraradices* Schenck & Smith (1982) (basionym of the actual name *Rhizophagus intraradices* C. Walker & A. Schüßler 2010) and *Glomus mosseae* (basionym of the actual name *Funneliformis mosseae* T.H. Nicolson & Gerd. C. Walker & A. Schüßler 2010) (MSY).

Sprouted offshoots of globe artichoke (*Cynara cardunculus* L. var. *scolymus* (L.) Fiori), var. Brindisino, were obtained from selected mother plants and transplanted in a greenhouse located at the University of Bari (41° 7′ 31″ N, 16° 52′ 0″ E) into pots containing peat pellets to induce the rooting. At the same time, they were inoculated as follows: for each pot, approximately 5 g of the sandy soil containing about 50 spores of MSE or 1.5 g of MSY were distributed immediately below the offshoots. Non-mycorrhizal plants were used as controls.

After 30 days, the rooted offshoots were transplanted into pots (volume=3.5 dm^3) containing a commercial substrate composed by 70% blond peat and 30% dark peat (Pindstrup Mosebrug A/S, Denmark) and grown for other 30 days before transplanting for the trial.

2.2. Site and soil characteristics

The experimental trial was conducted in Noci, Apulia region, Southern Italy, (40°79′18" N, 17°08′13" E; altitude 420 m a.s.l.) during the cropping cycle 2020–2021.

Each plantlet deriving from the greenhouse was transplanted in 1000 L pot containing a silty clay loam soil (Figure 1). The main soil chemical properties were determined according to the analytical methods by Sparks et al. (1996) and were the following: pH_{H2O}, 7.8; pH_{KCl}, 7.0, electrical conductivity, 2100 μS cm^{-1}; organic carbon, 7.8 g kg^{-1}; total nitrogen, 1.1 g kg^{-1}; available phosphorus, 49.5 mg kg^{-1}; total carbonates, 40.1 g kg^{-1}.

The experimental station is characterized by a typical Mediterranean climate, with air temperatures that drop below 0 °C in winter and exceed peaks of 40 °C in summer. The long-term average annual rainfall is about 590 mm, with precipitations unevenly distributed throughout the year and mainly concentrated in the period from October to April.

2.3. Treatments details and experimental design

The general experimental design was a split plot design with three irrigation treatments as main factors, and three AMF inoculations as sub factors. The three irrigation treatments were: treated wastewater coming from a wastewater treatment plant (WW), groundwater available on site (FW) and spiked FW (EW). The AMF inoculations consist in not inoculated artichoke as control (CON), MSE and MSY inoculated artichoke.

The combinations of irrigation treatments and AMF inoculations treatments were replicated in four blocks.

2.4. Crop management

A drip irrigation system was used as a single plastic pipe placed in the middle of each pot, with two drippers of 2 L h^{-1} flow rate. The soil moisture was detected using sensors (X-Farm) placed in

147 the top and middle soil layers per each treatment and replication. The irrigation started each time
148 the soil moisture reached the 25 % of the available water, and it was necessary a volume
149 corresponding to 250 m^3 ha^{-1} to restore soil moisture to 100%. From the end of April 2021 through
150 to June 2021 were applied three irrigations.

151 During the crop season, nitrogen and potassium were applied to all treatments two months after
152 transplanting at doses corresponding to 128 kg N ha^{-1} and 138 kg K_2O ha^{-1}, respectively. Four
153 months later, lateral offshoots were removed, leaving two shoots per plant, and 22 kg N ha^{-1}, 44 kg
154 P_2O_5 ha^{-1} and 32 kg K_2O ha^{-1} were applied.

155 Weed and pest management were carried out in accordance with local good agricultural practices
156 and were the same for all treatments.

157

158 **2.5. Chemicals and reagents**

159 The analytical standards (purity>99%) of carbamazepine, clarithromycin, climbazole,
160 fluconazole, ketoprofen, naproxen, sulfamethoxazole and trimethoprim were obtained from Lab
161 Instruments (Italy). These compounds were specifically selected due to their occurrence in
162 municipal wastewaters being not well removed during conventional biological treatments. In
163 general, CECs concentration in treated wastewaters ranged from low ng/L to low µg/L (Mordechay
164 et al. 2021; Riemenschneider et al. 2016; Rogowska et al. 2020; Tran et al. 2018). Standards were
165 used to prepare the multi compounds stock standard solution (1000 ppm). This solution was added
166 to water to achieve the concentration of 200 µg L^{-1} of each compound for obtaining EW. All
167 reagents used in the experiments as well as the solvents used for chromatographic analyses, i.e.,
168 acetonitrile (ACN) and formic acid, were analytical grade.

169

170 **2.6. Samples collection and characterization**

171 Water samples (FW, WW, and EW) were taken directly from drippers of the irrigation system.
172 All samples were placed into glass amber bottles and stored at 4 °C until the extraction and

quantification of the selected PhACs. Their concentration in water samples was determined following an online solid phase extraction (SPE) method whose analytical conditions (UPLC-QTOF/MS/MS) have been detailed elsewhere (Montagna et al. 2020). Table 1 shows the concentration of PhACs studied in each water used for irrigation.

2.7. Symbiotic parameters and morphological characteristics of the plants and the heads

To evaluate the effects of FW, EW and WW on the AMF, symbiotic parameters were monitored on artichoke roots at the time of transplant in field, before the first irrigation and after 10 days from the third irrigation. The staining of roots was carried out following Phillips and Hayman's method (1970). In details, a total of 10x1 cm root pieces per plant were chosen randomly from the staining root fragments and placed on a microscope slide. Ten microscope slides per each combination between mycorrhizal inoculum and water quality were prepared. The root fragments, mounted in a drop of glycerol, were observed using an optical microscope (Leica DMLB100). The frequency of mycorrhiza in the root system was evaluated as percentage according to Trouvelot et al. (1986).

The effect of MSE and MSY and FW, EW, and WW on the morphological parameters of the main artichoke heads (height, diameter, and weight) were also evaluated.

2.8. Pharmaceuticals extraction from soil and plants organs

The extraction of PhACs from soils were performed in accordance with the modified Quechers method reported by De Mastro et al. (2022b).

Before extraction of PhACs from plants, roots were gently hand washed with tap water to remove soil residues, subsequently rinsed with deionized water, and then gently blot dried with a paper towel. Roots, leaves, stems, and heads of artichoke were finely chopped and stored in 15-mL centrifuge tube in the dark at – 20 °C until extraction. Briefly, 2 g of plant material was placed in a 50 mL glass centrifuge tube and spiked with the relevant recovery surrogate. Water (6 mL) was

198 added to the centrifuge tubes followed by capping and vortexing for 1 min. Only the heads were left
199 to rest for 30 min after this step. Samples were then thoroughly wetted, and 10 mL ACN were
200 added to the centrifuge tubes and shaken by hand for 5 min. The method was followed by salting-
201 out step with citrate buffer (6 g $MgSO_4$, 1.5g Na Acetate). Tubes were vigorously shaken again by
202 hand for 5 min. Samples were subsequently centrifuged (5 min, 3700 rpm), which resulted in a
203 phase separation between the aqueous and organic solvents. The upper ACN layer (6 mL) was
204 transferred into 15-mL tubes for the clean-up step. Tubes containing 900 mg $MgSO_4$ + 150 mg PSA
205 for roots, or 900 mg $MgSO_4$ + 150 mg PSA + 150 mg C18 for leaves and stems, or 900 mg $MgSO_4$
206 + 150 mg PSA + 15 mg GCB for heads, were vortexed for 1 min. After centrifugation (5 min, 4000
207 rpm), the supernatant was filtered through a membrane filter (PVDF, 0.22 μm) and 1.5 mL were
208 transferred into a screw cap vial for LC-MS/MS analysis.
209
210 **2.9. Instruments and analytical determinations**
211 The quantification of PhACs in the extracts was performed using an Ultimate 3000 System
212 (Thermo Fisher Scientific) interfaced to a high-resolution mass spectrometer, TripleTOF 5600+
213 system (AB Sciex) equipped with a duo-spray ion source operated in positive electrospray
214 ionization mode. All the analyses were acquired with an acquisition method based on both full-scan
215 survey TOF MS and Information Dependent Acquisition (IDA) methods.
216 The chromatographic separation of analytes was achieved employing a ZORBAX Eclipse Plus
217 C18 column (150 x 2.1 mm, 1.8 μm) operating at a flow of 0.300 mL min^{-1}. The mobile phases
218 were 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). 100
219 μL of each extract were injected in the LC-MS system and eluted with the following gradient: 0−2
220 min, 2% solvent B; 2−3 min, linear from 2 to 20% solvent B; 3−17 min, linear from 20 to 100%
221 solvent B; 17−21 min, isocratic at 100% solvent B; 21−21.5 min, from 100 to 2% solvent B;
222 21.5−25 min, column reconditioning.

223 Each sample was spiked with an internal standard, carbamazepine-D10, at a level of 10 μg L^{-1}
224 and a reference calibration curve was injected in the range 0.1–10 μg L^{-1} (0.1, 0.2, 0.5, 1, 2, 5 and
225 10 μg L^{-1}) to quantify the PhACs.
226 AB Sciex software was used for data processing obtained by the high-resolution mass
227 spectrometry analysis. PeakView 2.2 and SciexOS 1.2 were employed for data interpretation and
228 for the determination of analytes concentration.
229
230 **2.10. Statistical data analysis**
231 All experimental data were conducted in triplicate and statistically analyzed using the R
232 software. In details, results were first tested for their normal distribution and homoscedasticity and,
233 successively, subjected to the Analysis of Variance and post-hoc test.
234
235 **3. Results and Discussion**
236 **3.1. Assessment of mycorrhizal frequency**
237 Mycorrhizal frequency evaluated on artichoke plants at the time of transplant and before the first
238 irrigation did not show significant differences between the two AMF used, as shown in Figure 2.
239 The control plants showed a mycorrhizal frequency lower than other treatments and it was due to
240 mycorrhizal fungi already present in soil.
241 In general, ten days after the third irrigation, the mycorrhizal frequency was significantly higher
242 in mycorrhizal plants with respect to the control ones (Table 2), confirming the high mycotrophy of
243 the plant species belonging to the *Asteraceae* family (Turrini et al. 2016 and 2018), such as *Cynara*
244 *cardunculus* var. *scolymus*. Regarding the artichoke, previous studies have recorded different
245 mycorrhizal colonization values depending on the cultivar, the propagation material used and the
246 mycorrhizal fungus (Campanelli et al. 2011; Ceccarelli et al. 2010; Ruta et al. 2018). For example,
247 *Septoglomus viscosum* showed already a high affinity with artichoke roots (Campanelli et al. 2013).
248 The percentage of mycorrhizal frequency was not affected by WW and FW, while the irrigation

249 with EW reduced that of MSE by about 35% (Table 2). This result suggests a higher susceptibility
250 of MSE to contaminated waters, as reported also by Sallach et al. (2021).

251 The highest percentage of mycorrhizal frequency was obtained with the WWxMSY combination
252 (65.3%), even if satisfactory mycorrhizal values higher than 50% were reached for all mycorrhizal
253 theses, except for the EWxMSE combination, where the percentage of the frequency was less than
254 40%. Finally, no significant difference was found for CON and MSY regardless of the water's
255 quality, while the EWxMSE combination reduced the mycorrhizal frequency by about one third
256 (38.3%) compared to the other two possible combinations for the same fungus (FWxMSE: 60%;
257 WWxMSE: 58.3%). In general, the better condition for the mycorrhizal symbiosis was the irrigation
258 with WW, probably due to the higher amount of nutrients such as total nitrogen or phosphorus
259 available for the fungi, as also reported by Chibuike (2013).

260 Table 3 shows the effects of FW, EW and WW and the mycorrhizal symbiosis on the
261 morphological parameters of artichoke. Although there was no statistically significant difference
262 among the theses, plants irrigated with FW showed a numerically slightly higher weight of heads
263 (152.2 g) with respect to these irrigated with EW (145.6 g) and WW (145.3 g). Regardless of the
264 different quality of the waters, the weight of the heads from mycorrhizal plants was numerically
265 higher compared to that of CON. The present results are in accordance with those found by Avio et
266 al. (2020) between the control plants and the plants inoculated with six different fungi.

267

268 **3.2. Pharmaceuticals residues in soils and plants**

269 Soils irrigated with FW did not show any PhACs, while the application of WW resulted in the
270 presence of the sole carbamazepine, whose concentration was 2.5 ng g^{-1}.

271 Figure 3 reports the average concentrations of each investigated pharmaceutical in soils irrigated
272 with EW. The two nonsteroidal anti-inflammatory drugs ketoprofen and naproxen were not found in
273 any soil, and MSE soil did not have sulfamethoxazole, trimethoprim and climbazole. The others
274 PhACs always showed a significantly higher concentration in control soils, followed by MSY and

MSE. The results can be possibly due to i) the intrinsic characteristics of each contaminant that determined their behaviour in soils (De Mastro et al. 2022a), ii) the easy degradability of many compounds by the microbial community or by photodegradation/oxidation processes (Ascar et al. 2017). In addition, the AMF utilized could have stimulated differently the release of root exudates and enzymes, and/or improved the soil structure. In fact, the quality of root exudates has been reported to influence the contaminants degradation (Joner et al. 2002; Nichols et al. 1997). It was noteworthy that in presence of major mycorrhizal frequency (MSY), the soil amount of PhACs was higher, contrary to what occurred in presence of plants with minor mycorrhizal frequency (MSE). In this regard, the two fungi occurring in the MSY did not show a synergistic effect with respect to the single fungus presents in MSE and influenced the root exudates modifying differently the microbial populations involved in the degradation of PhACs. Chibuike (2013) asserted that mycorrhiza did not always support the removal of organic contaminants from soil, and the amount of their removal depended on the mycorrhizal fungus employed (Arriagada et al. 2007). It is known that the benefit deriving from the plant-mycorrhiza association may differ depending on the plant and fungal species (Smith and Reed 2008). Regarding the artichoke, Avio et al. (2020) reported that different mycorrhizae differently modulated plant secondary metabolism, thus influencing the microbial community in soil able to degrade organic contaminants.

Carbamazepine and fluconazole were the sole pharmaceuticals found in artichoke organs (root, leaves and stems, heads) only when irrigated with EW (Figs. 4-6). MSE treated plants showed the best results, followed by MSY and control plants. Among the different parts of plants, the highest concentrations of carbamazepine and fluconazole were observed in leaves and stems. Mordechay et al. (2021) also reported that leaves contained the highest concentration of CECs when irrigated to treated wastewaters compared to fruits and tubers. In particular, the amount of carbamazepine found in control leaves was 10 times higher than that in mycorrhizal plants with MSE. In general, the uptake of different compounds by plants depends on the forms of their molecules and the degradation in soils (Kodešová et al. 2019). Compounds of intermediate lipophilicity ($0 < K_{ow} < 3$)

exhibit the highest translocation through plant compartments, with respect to compounds outside this range (Briggs et al. 1982). In this regard, fluconazole, carbamazepine, trimethoprim, and sulfamethoxazole were within the optimal range to ensure their translocation in plants, but the latter two show a high-water solubility that can determine their easy leaching from soils and can be rapidly degraded in soils with different characteristics (Koba et al. 2017; Kodešová et al. 2016).

In addition, carbamazepine possesses a non-ionic nature and low molecular weight (Kumar and Gupta 2016), and these characteristics allow carbamazepine to pass easily through plant root membranes and accumulate in leaves, as reported by many other studies (Goldstein et al. 2014; Hurtado et al. 2016; Malchi et al. 2014; Montemurro et al. 2017; Mordechay et al. 2018; Shenker et al. 2011; Winker et al. 2010; Wu et al. 2013).

4. Conclusions

Our study confirmed the hypothesis related to the positive correlation between mycorrhizal fungi and the reduction of PhACs concentration in soils and plants. In this regard, it is highly suggested to use mycorrhizal plants when the quality of the irrigation water concerns about the presence of organic contaminants. Apparently, the kind of fungus applied plays an important role in the reduction of the concentration of PhACs rather than the mycorrhizal frequency. In fact, in the present study, MSE showed the best results either in soils or plants.

Carbamazepine and fluconazole were the sole PhACs studied able to translocate and accumulate at different concentrations in the various organs of the artichoke only when irrigated with EW. Therefore, considering the concentration used to enrich the water and the real concentration of these two PhACs in WW, it is possible to exclude their entry into the food chain consuming artichokes irrigated with WW. Anyway, the risk assessment only based on monitoring of the parent compounds may lead to underestimation of the transformation products, which also need deep consideration. The identification and quantification of metabolites in edible plants, necessary for

better risk assessment regarding human health, will be the subject of future research. Finally, from an agronomic point of view, the application of WW did not influence the artichoke yield.

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Statements & Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

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Not applicable.

Consent to participate

Not applicable.

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Not applicable.

Author Contributions

Gennaro Brunetti, Giuseppe De Mastro, Francesco De Mastro, Claudia Ruta, Giuseppe Mascolo contributed to the study conception and design. Material preparation, data collection and analysis were performed by Francesco De Mastro, Andreina Traversa, Sapia Murgolo, Claudia Ruta, Cristina De Ceglie, Donato Stea. The first draft of the manuscript was written by Francesco De Mastro, Andreina Traversa, Sapia Murgolo, Claudia Ruta, Filomena Sannino and all authors commented on previous versions of the manuscript. The final manuscript was revised by Francesco De Mastro, Andreina Traversa, Claudio Cocozza. All authors read and approved the final manuscript.

Availability of data and materials

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures captions

Fig. 1 Experimental trial

Fig. 2 Mycorrhizal frequency (%) at the time of the transplant and before the first irrigation

Fig. 3 Average concentration (ng g^{-1}) of the compounds detected in EW irrigated soil (n = 3)

Fig. 4 Average concentration (ng g^{-1}) of the compounds detected in the roots of plants irrigated with EW (n = 3)

Fig. 5 Average concentration (ng g^{-1}) of the compounds detected in the leaves and stems of plants irrigated with EW (n = 3)

Fig. 6 Average concentration (ng g^{-1}) of the compounds detected in the heads of plants irrigated with EW (n = 3)

Figure 1

Figure 2

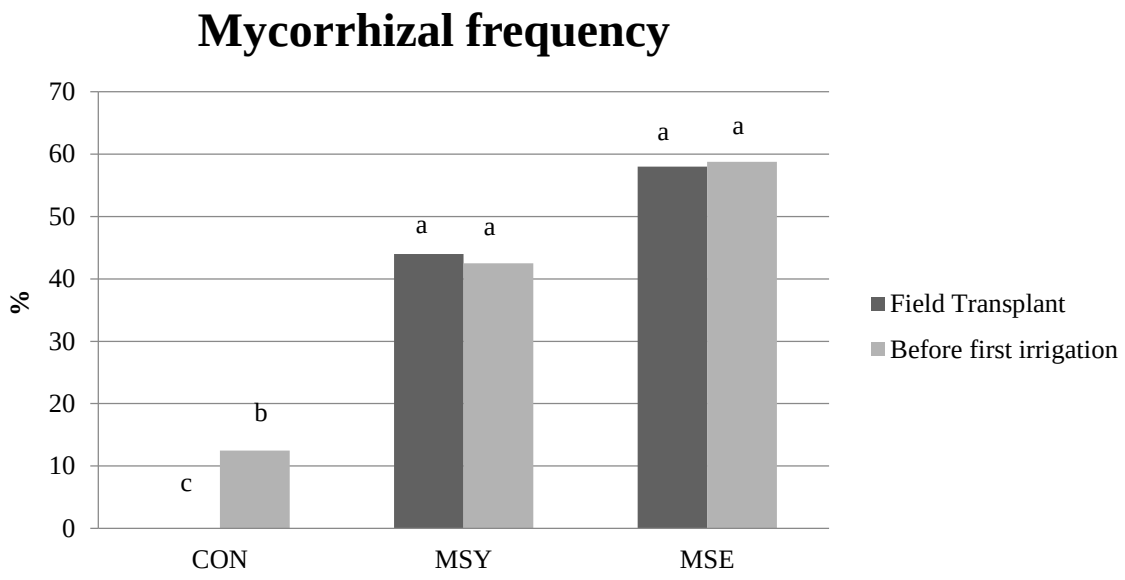

Figure 2

Figure 3

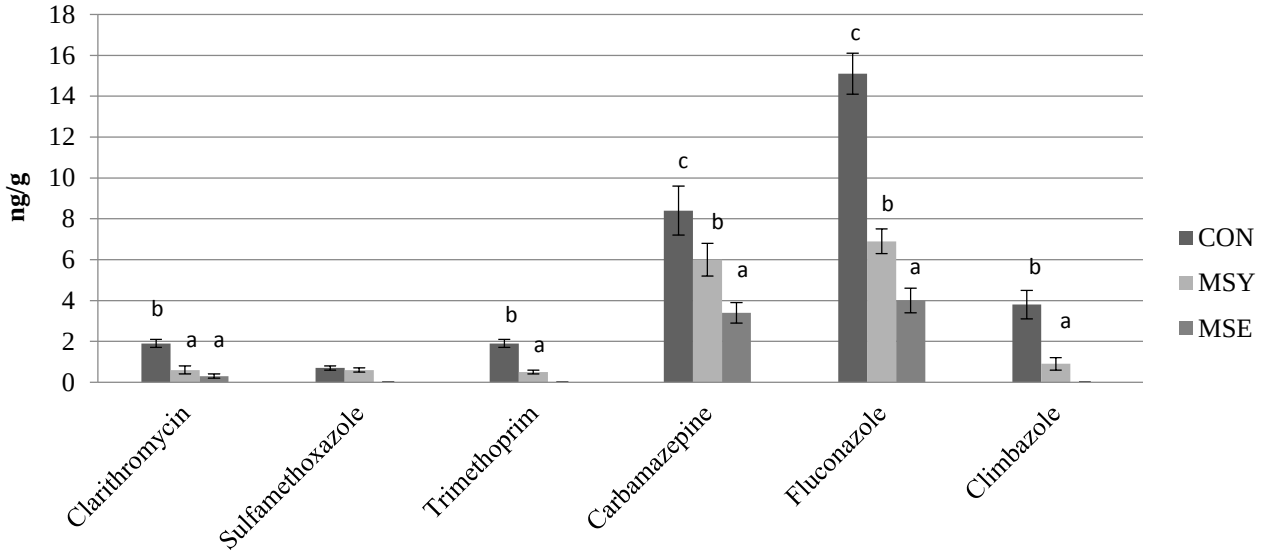

Figure 3

Figure 4

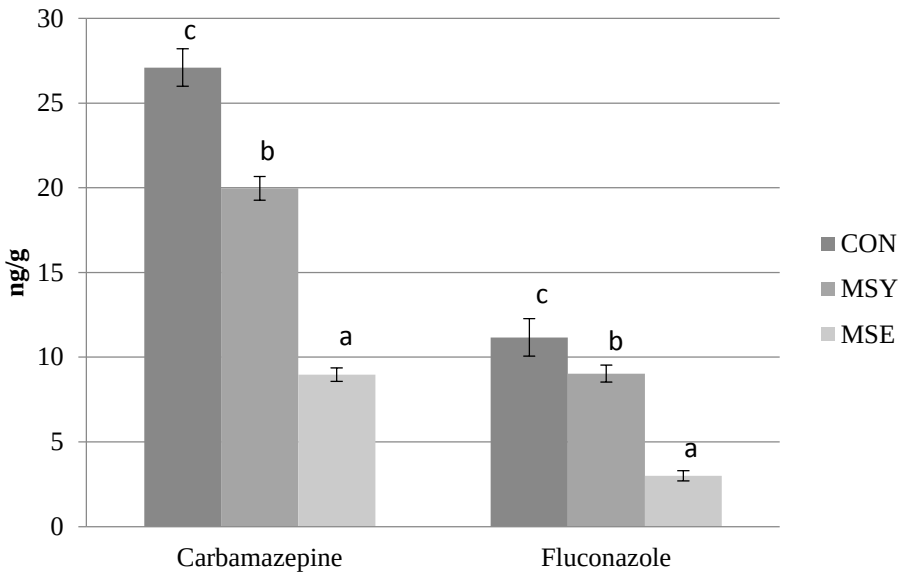

Figure 4

Figure 5

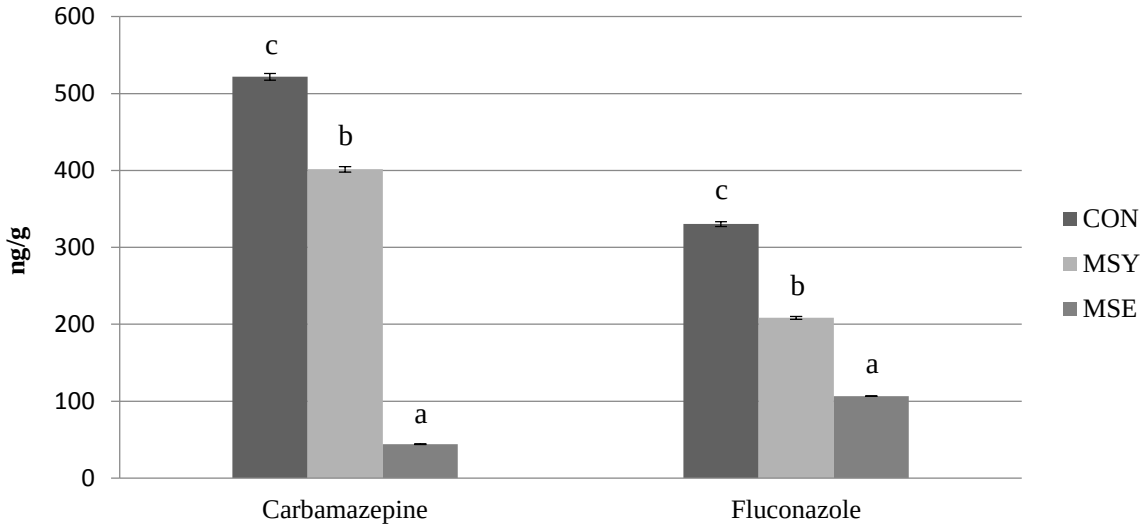

Figure 5

Figure 6

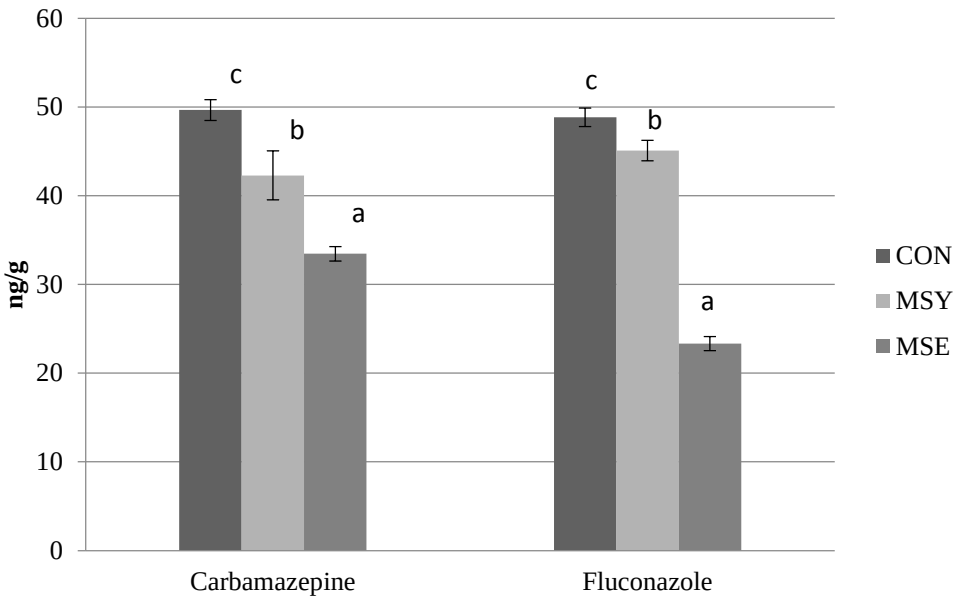

Figure 6

Table 1

Table 1. Average concentration (µg L^{-1}) of the PhACs detected in FW, WW and EW (n = 3)

Pharmaceuticals	**Category**	**LOQ**	**FW**	**WW**	**EW**
		µg/L	µg/L		
Clarithromycin	antibiotic	0.01	<LOQ	0.08 ± 0.001	223.8 ± 4.4
Sulfamethoxazole	antibiotic	0.05	<LOQ	<LOQ	187.9 ± 1.8
Trimethoprim	antibiotic	0.01	<LOQ	<LOQ	192.3 ± 7.6
Ketoprofen	anti-inflammatory	0.01	<LOQ	0.2 ± 0.01	48.9 ± 8.2
Carbamazepine	antidepressant	0.01	<LOQ	0.54 ± 0.04	214.5 ± 11.0
Fluconazole	antifungal	0.01	<LOQ	0.07 ± 0.002	232.6 ± 12.2
Climbazole	antifungal	0.01	<LOQ	0.03 ± 0.001	197.2 ±10.8
Naproxen	anti-inflammatory	0.1	<LOQ	0.21 ± 0.02	211.2 ± 20.2

Table 2. Effect of both mycorrhizal inocula and irrigation with FW, EW and WW on the frequency (%) of mycorrhiza evaluated 10 days after the third irrigation intervention.

Water quality	Inoculum	Frequency (%)
EW	CON	6.7 d
	MSE	38.3 abc
	MSY	51.6 ab
Average EW		**32.2**
FW	CON	15.0 cd
	MSE	60.0 ab
	MSY	55.0 ab
Average FW		**43.3**
WW	CON	25.0 bcd
	MSE	58.3 ab
	MSY	65.3 a
Average WW		**50.5**

Different letters within the column indicate significant differences according to Tukey's test ($P \leq 0.05$)

Table 3. Effect of of both mycorrhizal inocula and irrigation with FW, EW and WW on the morphological parameters of artichoke heads at the primary head harvesting time during the first growing season (2020-2021).

Water quality	Inoculum	Height (cm)	Diameter (cm)	Weight (g)
EW	CON	9.7	6.8	131.0
	MSE	7.7	7.4	157.0
	MSY	7.7	6.6	148.7
Average EW		**8.4**	**6.9**	**145.6**
FW	CON	7.7	6.3	154.0
	MSE	8.0	7.4	162.0
	MSY	9.5	7.5	158.5
Average FW		**8.4**	**7.0**	**152.2**
WW	CON	8.7	6.8	142.0
	MSE	8.5	6.7	134.5
	MSY	8.8	7.3	159.3
Average WW		**8.7**	**6.9**	**145.3**