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Spread of organic micropollutants and per-and polyfluoroalkyl substances (PFAS) to farmlands when irrigated with municipality wastewater

Spridning av organiska mikroföreningar och och per- och polyfluoralkyl substanser (PFAS) till jordbruk vid bevattning med kommunalt avloppsvatten

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Sammanfattning

Återanvändning av avloppsvatten för jordbruksbevattning är ett sätt att minska efterfrågan på sötvatten, men det kan också introducera läkemedel, personliga hygienprodukter och andra kemikalier i jordbrukets ekosystem och livsmedelskedja. Syftet med denna studie var att mäta förekomsten av en rad potentiellt skadliga organiska mikroföroreningar (OMP), såsom läkemedel, personliga hygienprodukter, industrikemikalier, bekämpningsmedel och per- och polyfluoralkylsubstanser (PFAS) i avloppsvatten som används för bevattning och i den mottagande jordbruksmarken. Bevattningsvatten bestående av renat avloppsvatten vid två anläggningar på Gotland provtogs vid fyra tillfällen under juli och augusti 2021. Dessutom togs prover på jord och daggmaskar i september 2021 efter skörd från två åkrar bevattnade med avloppsvattnet samt från en åker som inte hade bevattnats alls. Potatis som fortfarande var i marken togs från ett av de bevattnade fälten.

Ett brett spektrum av OMP-koncentrationer (ng/l till ug/l) hittades i bevattningsvattnet, och totalt upptäcktes 35 av 77 analyserade OMP. Totalt 19 av 24 olika PFAS detekterades i bevattningsvattnet i intervallet 0,1-1000 ng/l. Extraherbart organiskt fluor (EOF) och totala oxiderbara prekursorer (TOP) mättes även i vattenproverna. Intervallet för EOF var 366-507 ng/l och endast två av totalt åtta bevattningsvattenprover visade en liten ökning av perfluoroktansyra (PFOA) och perfluorhexansyra (PFHxA) efter oxidation.

Ingen av OMP:erna i denna studie upptäcktes i något av jordproverna. Perfluoroktansulfonsyra (PFOS) hittades över detektionsgränsen i jord från båda bevattnade fälten (0,14-0,17 ng/ d.w.) men låg under detektionsgränsen i jord från referensplatsen. Perfluorbutansyra (PFBA) hittades i låga nivåer men över detektionsgränsen i ett av de bevattnade fälten (0,22

ng/g d.w.) och den närliggande referensplatsen (0,27 ng/g d.w.). EOF-analysen av jord resulterade inte i någon ytterligare information då detektionsgränsen var relativt hög (280 ng/g).

Av 77 analyserade OMP:er hittades endast fem OMP:er (sotalol, oxazepam, metformin, etylparaben och venlafaxin) i daggmaskar i koncentrationsintervallet 8,4-160 ng/g d.w. Mellan fem och tio PFAS upptäcktes i daggmaskar från de två bevattnade fälten (19-35 ng/g d.w. för summan PFAS) och sju PFAS hittades i maskar från referensplatsen med en summakoncentration på 69 ng/g d.w. Endast två OMP-föreningar (6,6 ng/g d.w. för metformin och 340 ng/g d.w. för BAM) hittades i potatis som samlats in från en bevattnad åker, och ingen av de analyserade PFAS kunde detekteras.

Den aktuella studien kunde bara upptäcka ett fåtal PFAS och andra OMP i jord och potatis från marken som bevattnats med avloppsvatten. Bioackumulering av fem OMP och upp till tio PFAS påvisades i daggmaskar på den bevattnade jordbruksmarken men även på referensplatsen. Således kunde ingen påverkan av avloppsvatten på marken eller daggmask påvisas. Detta var dock en begränsad studie och det kan inte uteslutas upptag av OMP till ätbara växter eller läckage till grundvatten.

Summary

The reuse of wastewater for agricultural irrigation is one way to reduce the demand for freshwater but it can also introduce pharmaceuticals, personal care products, and other chemicals into the agricultural ecosystem and food chain. The aim of this study was to measure a range of potentially harmful organic micropollutants (OMPs), such as pharmaceuticals, personal care products, industrial chemicals, pesticides, and per- and polyfluoroalkyl substances (PFAS), in wastewater effluent used for irrigation and in the receiving farmland. Four sampling occasions of effluent water at two wastewater treatment plants in Gotland were conducted during July and August 2021. In addition, soil and earthworms were sampled in September 2021 after harvest from two fields irrigated with the monitored wastewater and one field that had not been irrigated that season. Potatoes still in the ground were sampled from one of the irrigated fields.

A wide range of OMP concentrations (ng/L to ug/L) were found in the effluent water, and in total 35 of 77 analyzed OMPs were detected. A total of 19 out of 24 different PFAS were detected in the effluent samples in the range of 0.1-1000 ng/L. Extractable organofluorine (EOF) and total oxidizable precursors (TOP) were measured in water samples in addition to target PFAS. The range for EOF in effluent samples were 366-507 ng/L and only two samples out of the total eight effluent samples showed a small increase in concentrations of perfluorooctanoic acid (PFOA) and perfluorohexanoic acid (PFHxA) after oxidation.

None of the OMPs in this study was detected in any of the soil samples. Perfluorooctane sulfonic acid (PFOS) was found over the detection limit in soil from both irrigated fields (0.14-0.17 ng/g d.w.) but was below the detection limit at the reference site. Perfluorobutanoic acid (PFBA) was found at low levels closely above the detection limit in one of the irrigated fields (0.22 ng/g d.w.) and the nearby reference site (0.27 ng/g d.w.). The EOF analysis of soil did not result in any further information due to an elevated detection limit (280 ng/g d.w.).

Out of 77 analyzed OMPs, only five OMPs (sotalol, oxazepam, metformin, ethylparaben, and venlafaxine) were found in earthworms in the concentration range 8.4-160 ng/g d.w. Between five and ten PFAS were detected in earthworms from the two irrigated fields (19-35 ng/g d.w. for ∑PFAS) and seven PFAS were found in worms from the reference site with ∑PFAS concentration of 69 ng/g d.w.

Only two OMP compounds (6.6 ng/g d.w. for metformin and 340 ng/g d.w. for BAM) were found in potatoes collected from one irrigated field, and none of the PFAS could be detected.

The present study could detect only a few PFAS and other OMPs in soil and potatoes from the land irrigated with wastewater effluent. Bioaccumulation of five OMPs and up to ten PFAS was shown in earthworms at the irrigated farmland but also at the reference site. Thus, this study could not reveal any impact of irrigated wastewater to the soil or earthworm. However, this was a limited study and it cannot be excluded the uptake of OMPs to edible plants or leaching into groundwater.

1. Introduction

Reclaimed water (e.g., municipal sewage or domestic sewage that can be reused for non-drinkable water applications) is an important alternative to the traditional water supply [1]. The reuse of reclaimed wastewater for agricultural irrigation has attracted much attention as a way to reduce the demand for freshwater supplies [1-3]. One primary concern associated with reusing wastewater in agricultural irrigation is the introduction of pharmaceuticals, personal care products and other chemicals into the agricultural ecosystem and food chain. It can be potentially harmful to humans consuming the agricultural products. Currently, a range of potentially harmful organic micropollutants (OMPs), such as pharmaceuticals, personal care products, industrial chemicals and pesticides, were found in the reclaimed wastewater [2, 3]. Previous studies have demonstrated that the removal of OMPs in wastewater can vary depending on the implemented treatment processes [4-6]. OMPs detected in the reclaimed wastewater can lead to a variety of concerns for human and ecosystem health, including endocrine disruption, antibiotic resistance, and developmental disorders [2, 7, 8].

Per- and polyfluoroalkyl substances (PFAS) are anthropogenic chemicals having a perfluoroalkyl moiety (C_nF_{2n+1}) and often also different polar groups. PFAS has been monitored in sewage from Swedish wastewater treatment plants (WWTPs) and different classes of PFAS have been found in effluent and sludge [9]. As for OMPs, most commercial treatment processes show poor removal efficiency for many PFAS and increased levels are often seen in effluent compared to influent wastewater due to transformation of precursor compounds in the WWTP. However, there are thousands of different PFAS used on the global market with a large variation of structures and properties. To get an indication of the total amount of PFAS, analysis of extractable organofluorine (EOF) using combustion-ion-chromatography (CIC) may provide an assessment of other compounds that are not monitored.

Recently, a great number of scientific publications enlightened the chronic contamination of aquatic environments by OMPs [4, 10]. OMPs and PFAS are continuously excreted or discarded into the sewer systems as the unaltered parent compounds or their metabolites/transformation products. Subsequently, they often end up in the environmental waters, as a consequence of incomplete elimination by WWTPs [10]. Assessment of contamination levels and potential novel contaminants must address different aspects of this environmental problem.

The general objective of this study is to evaluate the presence of OMPs including pharmaceuticals, personal care products, industrial chemicals and pesticides, together with PFAS in the irrigation water-soil-earthworms-crops (potato) system, and their potential to be bioconcentrate into potato and earthworms.

2. Material and methods

2.1 Target analytes and chemicals

2.1.1 OMPs

All analytical standards used for analysis were of high purity grade (>95%). Native standards (*n*=77) were acquired from Sigma-Aldrich (Sweden). Isotopically labeled standards (IS) (*n*=18) for the target compounds were obtained from Wellington Laboratories (Canada), Teknolab AB (Kungsbacka, Sweden), Sigma-Aldrich, and Toronto Research Chemicals (Toronto, Canada). Detailed information about internal standards (IS) and native standards can be found elsewhere [11]. A list of the 77 OMPs included in the study, is given in Appendix table A1.1.

Ultrapure water was generated by a Milli-Q (MQ) Advantage Ultrapure Water purification system and filtered through a 0.22 µm Millipak Express membrane and an LC-Pak polishing unit (Merk Millipore, Billercia, MA). Methanol, acetonitrile, formic acid and ammonia of high analytical grade were acquired from Sigma-Aldrich (Sweden).

2.1.2 PFAS

A list of the 24 PFAS included in the study, together with abbreviations and full names, is given in Appendix table A1.2. Native (n=24) and isotopically labeled internal (n=15) standards were purchased from Wellington Laboratories (Guelph, Canada), with the exceptions of trifluoroacetate (TFA; from Merck KGaA; Darmstadt, Germany) and perfluoropropanoic acid (PFPrA; from Sigma-Aldrich). Water was laboratory produced 18.2 MΩ.cm Type 1 ultrapure water. Methanol (LC-MS grade) and ammonium hydroxide was purchased from Fisher Scientific (Hampton, United States). Potassium persulfate, ammonium acetate, sodium carbonate and sodium hydroxide were from Sigma-Aldrich (St Luis, MO, USA).

2.2 Sampling

In 2021, sampling campaigns were carried out to collect: i) samples of WWTP effluent water used for irrigation (*n* = 8), ii) soil samples (*n* = 3 pools), iii) earthworms (*n* = 3 pools) and iv) potato samples (*n* = 1 pool). The irrigation water samples were collected in July and August during the irrigation period. Soil, earthworms and potato samples were sampled by the end of the irrigation period in September.

WWTP effluent used as irrigation water was taken by the local staff from two wastewater treatment facilities in Gotland, in the area of Hemse and Roma. Pre-cleaned high-density polyethylene bottles, disposable gloves and ethanol/water cleaning liquid to rinse any sample equipment needed was sent to the facilities. A total of four grab samples, 2L each, were taken with 1-2 weeks interval, and was sent on icepacks with courier to Örebro University. One empty bottle was opened and placed next to the sampling location during sampling and was analyzed as a field blank.

Soil was taken at two fields that had received irrigation water from the wastewater treatment plants, one in the area of Hemse and the other one in Roma. Both fields had been harvested. One additional field in the area of Hemse, that had not been irrigated at all, was included as reference. A 4-zone

based sampling of the soil surface down to 17 cm were done at each site using probes and polyethylene bags. Subsamples for each site were pooled and kept at 8 degrees Celsius.

Earthworms were collected in the same zones as the soil. They were kept in soil and polypropylene containers during transport to the laboratory. Thereafter, they were kept on cellulose filters for 3-5 days before they were frozen and homogenized into one pooled sample for each sampling site.

Potatoes that were not harvested yet were taken from the soil in a small area of the field in Roma. They were kept under aluminum foil in a high-density polypropylene container at 8 degrees Celsius until analysis.

2.3 Sample preparation

2.3.1 OMPs

Procedures for preparation of water samples for instrument analysis were done as described previously by Sörengård et al. [12]. Briefly, water samples (200 mL) including blanks (n = 2) were extracted by solid-phase extraction (SPE) using Oasis HLB-cartridges (6 mL, 200 mg, 30 µm).

The soil samples were prepared using an ultrasonic-based solvent approach, for which detailed information can be found elsewhere [13]. Briefly, the whole samples were air-dried overnight in a clean fume hood. Before extraction, IS mixture (c = 10 ng/g d.w. sample) was added to 2 g dry soil sample. Then 4 mL of acetonitrile and water (1/1, v/v, 0.1% formic acid) were added to the air-dried soil and the samples were ultrasonicated for 15 min. The supernatant was filtered through a syringe filter (0.45 µm, regenerated cellulose, VWR, Sweden) into 10-mL vials. The step was repeated with a second extraction solvent mixture (acetonitrile, 2-propanol, and water (3/3/4 v/v/v with 0.1% formic acid)). The two supernatants were combined, mixed well, and 1 mL of the extract was used for analysis.

The earthworms samples were prepared according to a previously established protocol [14].

The potatoes samples were extracted by a validated in-house method [15, 16].

2.3.2 PFAS

2.3.2.1 Irrigation water

Irrigation water samples were filtered through glass fiber filters (Whatman Grade GF/B) prior to extraction. Filters were pre-baked at 450 °C for at least 12 h. Extraction of the water was done by solid-phase extraction using a method adopted from Miyake et al. [17] with some modifications. Briefly, 250 mL sample was adjusted to pH 4 using glacial acetic acid and extracted in duplicates using Oasis WAX cartridges (Waters Corporation; 150 mg, 6 cc). One replicate, intended for target PFAS quantification was fortified with labeled internal standards before extraction. The second replicate was not fortified and was used for EOF analysis and total oxidizable precursor assay (TOPA). The final extract from the second replicate was split after extraction, one part was

fortified with labeled internal standards for fluorine mass balance and the second part was divided for i) EOF analysis and ii) further oxidation using TOPA.

Oxidation (TOPA) of 0.1 mL extract was performed after addition of oxidation control standard (labeled FOSA). The reaction was performed with sodium hydroxide and persulfate at 85 degrees Celsius for six hours. The target analytes were separated from the reaction solution before instrumental analysis by using solid-phase extraction, as already described. Target analyte data after oxidation was compared to target analyte data before oxidation.

2.3.2.2 Solid samples

Pooled soil (sieved <2 mm), earthworm, and potato samples were freeze-dried, and three sub-samples were taken for chemical analysis. For each sub-sample, two replicates of soil, earthworms and potatoes (0.2-0.5 g) were first digested by sodium hydroxide and thereafter extracted with methanol. Further clean-up using graphitized carbon (ENVI-Carb, Supelco) was performed. One replicate was fortified with labeled internal standards before extraction and was intended for target PFAS quantification. The final extract from the second replicate was went through an additional clean-up using Oasis WAX SPE (Waters Corporation, Manchester, UK) and was thereafter split into two parts, one part was fortified with labeled internal standards for target PFAS analysis intended for fluorine mass balance and the second part was taken for EOF analysis.

2.4 Instrumental analysis and quality control

2.4.1 OMPs

The water, soil, earthworms and potatoes samples were analyzed by a DIONEX UltiMate 3000 ultra-high pressure liquid chromatography (UPLC) system (Thermo Scientific, Waltham, MA, USA) coupled to a triple quadrupole mass spectrometer (MS/MS) (TSQ QUANTIVA, Thermo Scientific, Waltham, MA, USA).

An Acquity UPLC BEH-C18 column (2.1 x 50 mm, 1.7 µm particle size; Waters Corporation, Manchester, UK) was used as an analytical column for chromatographic separation. Heated electrospray ionization (H-ESI) was used to ionize the target compounds. The spray voltage was set to static: positive ion (V) 3500. Nitrogen (purity >99.999%) was used as sheath gas (50 arbitrary units), auxiliary gas (15 arbitrary units), and sweep gas (2 arbitrary units). The vaporizer was heated to 400°C and the capillary to 325°C.

The mobile phase consisted of Milli-Q water with the addition of 5 mM ammonium acetate (phase A) and acetonitrile (phase B). The same linear gradient was used in both ionization modes, with a flow rate of 0.5 mL/min. The gradient started at 2% of phase B and increased to 99% from 0.5 min to 10.0 min. This composition of the mobile phase was maintained for 3 minutes, until 13.0 min, after which it returned to initial conditions at 13.1 min. Such composition was maintained until the end of the analytical run, which took 15 minutes.

The chromatography data acquisition was performed in positive and negative mode using selected-reaction monitoring. Xcalibur software (Thermo Fisher Scientific, San Jose, CA, USA) was used for optimizing the instrument methods and running samples. The data obtained were evaluated using TraceFinderTM 3.3. software (Thermo Fisher).

Quality controls for water, soil, earthworms and potato samples included analysis of blanks, limit of quantification (LOQ), matrix effect, and recovery. The concentration ranges in calibration curves for substances were in 0.01-1000 ng/L water, 0.01-100 ng/g sediment, 0.01-1000 ng/g earthworms and 0.01-500 ng/g potato samples. The method blanks were prepared and extracted in the same way as the samples and no target analytes were detected in method blanks. Matrix-matched standards were used to assess the matrix effect and were prepared from sample extract spiked with ISs and native OMPs.

2.4.2 PFAS

2.4.2.1 Target PFAS

Targeted analysis was carried out on an Acquity UPLC coupled to a Xevo TQ-S triple quadrupole mass spectrometer (Waters Corporation), equipped with a BEH C18 column (1.7 μm, 100 × 2.1 mm, Waters Corporation, Manchester, UK). The mobile phase consisted of 2mM ammonium acetate in water and methanol. An isolator column was inserted after the solvent mixer before the injector to separate any potential contamination from the UPLC system from the injected sample. The system was operated in negative electrospray ionization (ESI-) mode. The source and desolvation temperatures were set at 150 °C and 400 °C, respectively. The desolvation and cone gas flows (nitrogen) were set at 800 L/h and 150 L/h, respectively. The capillary voltage was set at 0.7 kV. Quantification was performed using labeled internal standards and an eight-point linear calibration curve ranging from 0.02 to 40 ng/mL.

Analysis of TFA, PFPrA, TFMS, PFEtS, PFPrS was carried out by supercritical fluid chromatography (SFC) coupled to tandem mass spectrometry (MS/MS) (Acquity Ultra Performance Convergence Chromatograph and Xevo TQ-S micro, Waters Corporation, Manchester, UK) operated in negative electrospray ionization mode. An SFC Torus DIOL column (1.7 μm, 150 × 3.0 mm) (Waters Corporation, Manchester, UK) maintained at 50 °C was used to achieve chromatographic separation. The mobile phase consisted of carbon dioxide and 0.1% ammonium hydroxide in methanol. The flow rate was 1.2 mL/ min and the active back pressure regulator (ABPR) for carbon dioxide was kept at 2000 psi. The source and desolvation temperatures were set at 150 °C and 350 °C, respectively. The desolvation and cone gas flows (nitrogen) were set at 650 L/h and 1 L/h, respectively. The capillary voltage was set at 2 kV.

Quantification was performed using labeled internal standards and a four-point linear calibration curve ranging from 2 to 50 ng/mL. Each batch of samples contained procedural blank and a fortified sample. The procedural blank was used for calculating limit of detection, together with

the lowest concentration standard in the calibration curve. Recovery of all analytes were monitored in the fortified sample, together with the internal standard recoveries.

2.4.2.2 EOF

A combustion ion chromatography (CIC) system with a combustion module from Analytik Jena, (Germany), and an ion chromatograph from Metrohm (Switzerland) was used to quantify extractable organofluorine (EOF). The anions were separated with an ion exchange column (Metrosep A Supp 5–150/4), carbonate buffer (64 mmol/L sodium carbonate and 20 mmol/L sodium bicarbonate) as eluent and isocratic elution. The autosampler injected 100 µL of the extract on a quartz boat. The boat was inserted into the oven (1000–1050 °C) under a flow of oxygen (300 mL/min), argon (100 mL/min), and argon mixed with water vapor (100 mL/min) under hydropyrolytic conditions monitored by a flame sensor followed by 2 minutes of post-combustion time with the flow of oxygen (400 mL) only. The hydrogen fluoride (HF) formed during combustion was absorbed in ultrapure water (in the absorber module). The F^- concentration was measured via conductivity.

The concentration of EOF is expressed in ng/g (solid samples) or ng/L (irrigation water) and represents the organofluorine content. The sample treatment methods ability to remove inorganic fluoride has been discussed elsewhere [25]. A five-point calibration curve of 50-1000 µg/L using PFOA as standard was used for quantification. Empty boat combustions were made before and after each sample and the average background was subtracted from the sample signal. Procedural blanks were run for monitoring background contamination and PFAS-fortified samples were included for soil, earthworms and potatoes to assess the recovery of organofluorine.

3. Results and discussion

3.1 Irrigation water

3.1.1 OMPs

In the present study, 35 out of 77 analysed OMPs including pharmaceuticals, personal care products, industrial chemicals and pesticides were detected in treated wastewater effluent, which was used for irrigation (Figure 1, Table A2.1 in appendix). Mean concentrations detected for the compounds ranged from ng/L to µg/L in irrigated water samples. Given this wide range of concentrations, carbamazepine, lamotrigine, metformin, bicalutamide and caffeine were presented in a high concentration (up to 460 ng/L), Figure 1. The compound composition and concentration ranges varied between sampling days and sampling locations. The highest concentrations of studied compounds were detected in sampling location Hemse, except of last sampling event in August.

Variation in effluent concentrations can be associated with different WWTP characteristics that can influence OMPs removal during wastewater treatment. WWTP operational factors such as hydraulic residence times, flow rates, sludge age and wastewater characteristics are factors that vary between WWTPs and can influence OMPs removal.

These results are in the same concentration range as previously reported for antidepressants and antiepileptic drugs in Swedish WWTPs and surface water [4, 18]. Golovko et al. [4] performed a screening study in Sweden for 164 OMPs in wastewater samples were collected and analyzed; similar concentration levels were found for most of pharmaceuticals compared to this study.

Metformin is by far the most often prescribed antidiabetic drug worldwide and in Sweden and it is usually taken in relatively high doses of 0.5–2 g/day [19]. It was shown that metformin is not completely metabolized in the human body [20] and it is excreted unchanged and therefore released into the environment via wastewaters. As a result, metformin is a drug with one of the highest environmental emissions. It is often detected in WWTP influents and fresh waters [21, 22]. It was suggested that carbamazepine and caffeine are suitable wastewater indicators of river water, the presence of these compounds in river water is a strong indication of wastewater contamination [23].

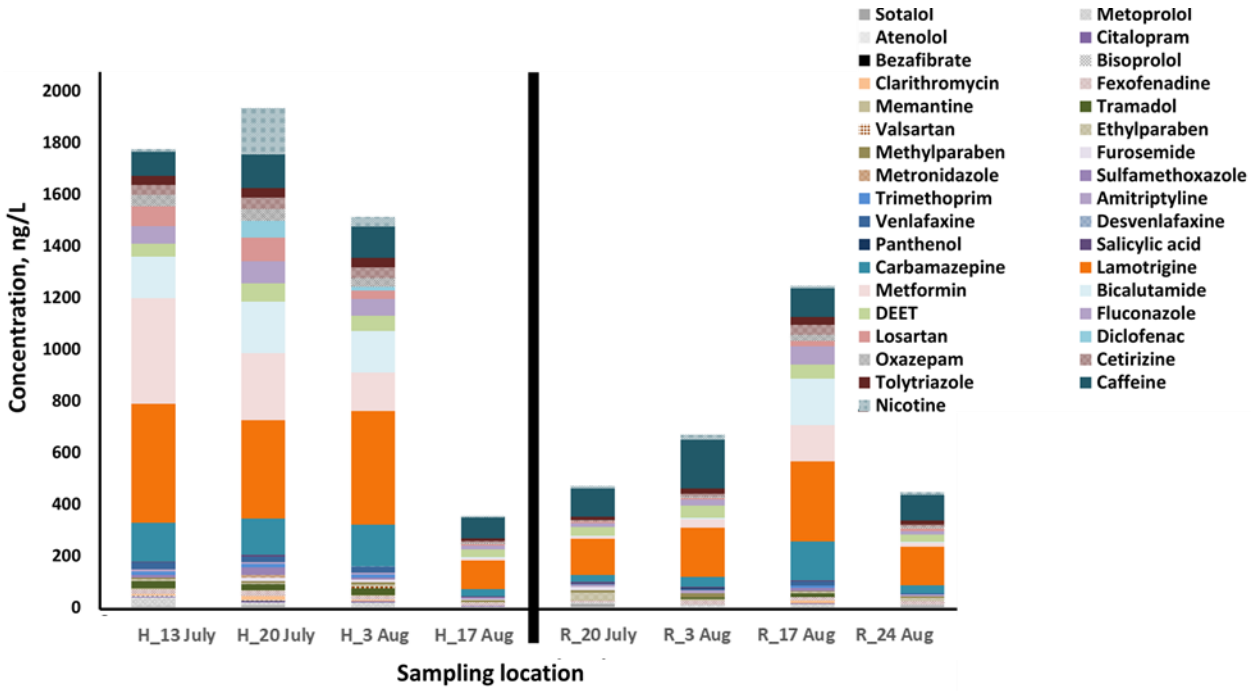

Figure 1. Occurrence of detected OMPs in irrigation water from two different sampling locations in July and August 2021. H - Hemse, R - Roma.

3.1.2 PFAS

In total, 19 different PFAS were detected in the irrigation water samples, although three were only detected in few samples and close to detection limit (Figure 2, Table A2.2 in appendix). In contrast to OMPs, the highest sum of PFAS concentration was found in Roma. The single compound that dominated the sum of PFAS was trifluoroacetate (TFA), 846-1011 ng/L in Hemse, and 1099-1390 ng/L in Roma. TFA has already been widely reported in surface water and rainwater at high levels [24]. Excluding TFA, the remaining PFAS pattern of perfluoroalkyl sulfonic acids (PFSA) and perfluoroalkyl carboxylic acids (PFCA) was similar between the two locations with slightly higher levels at Hemse (Figure 3). The short carbon chain acids dominated, more specifically TFMS, PFPrA, PFBA, and PFHxA, but also PFOA was among the most prominent compounds found.

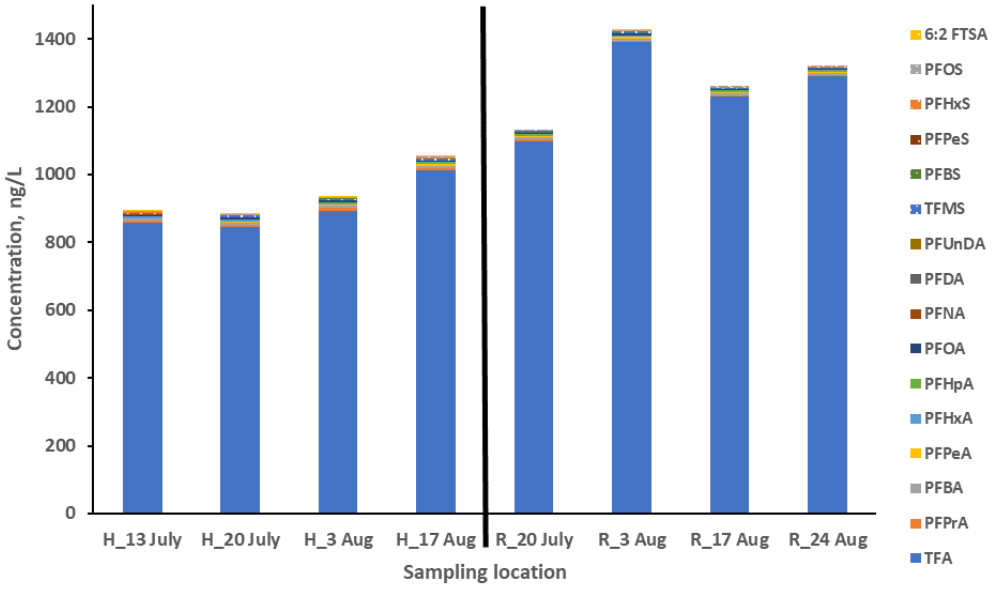

Figure 2. Occurrence of detected PFAS in irrigation water from two different sampling locations in July and August 2021. H - Hemse, R - Roma.

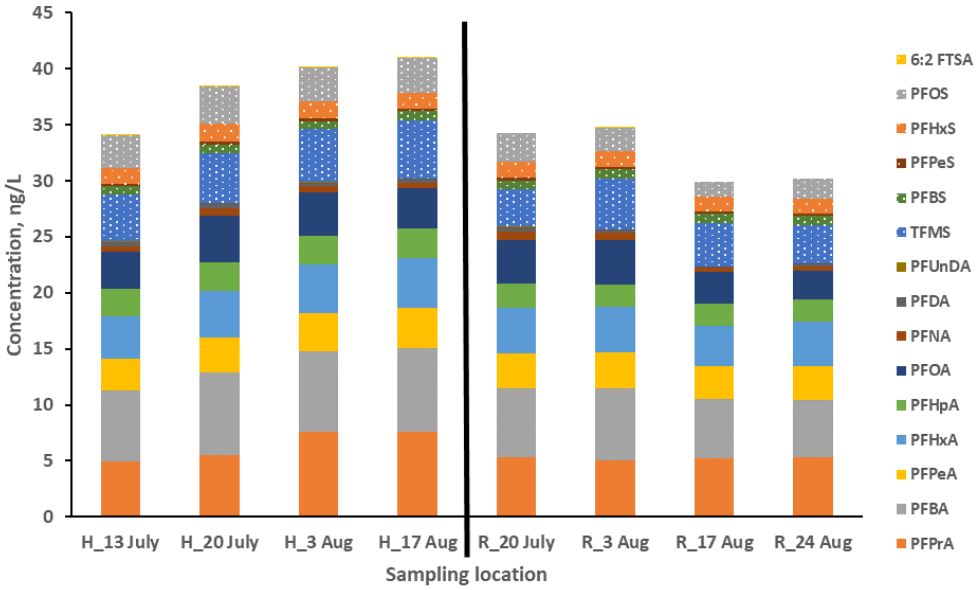

Figure 3. Occurrence of detected PFAS, excluding TFA, in irrigation water from two different sampling locations in July and August 2021. H - Hemse, R - Roma.

EOF analysis resulted in fluoride levels between 366-390 ng/L in Roma and 444-507 ng/L in Hemse (Figure 4). For comparison, a pooled effluent sample from 5 different Swedish wastewater treatment plants sampled from 2012 to 2018 was reported to contain 445-785 ng/L EOF (reported by three different laboratories) [25]. The biggest difference between the two locations was that target PFAS in the EOF extract, including TFA, after conversion to fluorine equivalents constituted 78-91% of EOF for Roma, while the same range for Hemse was 46-62%.

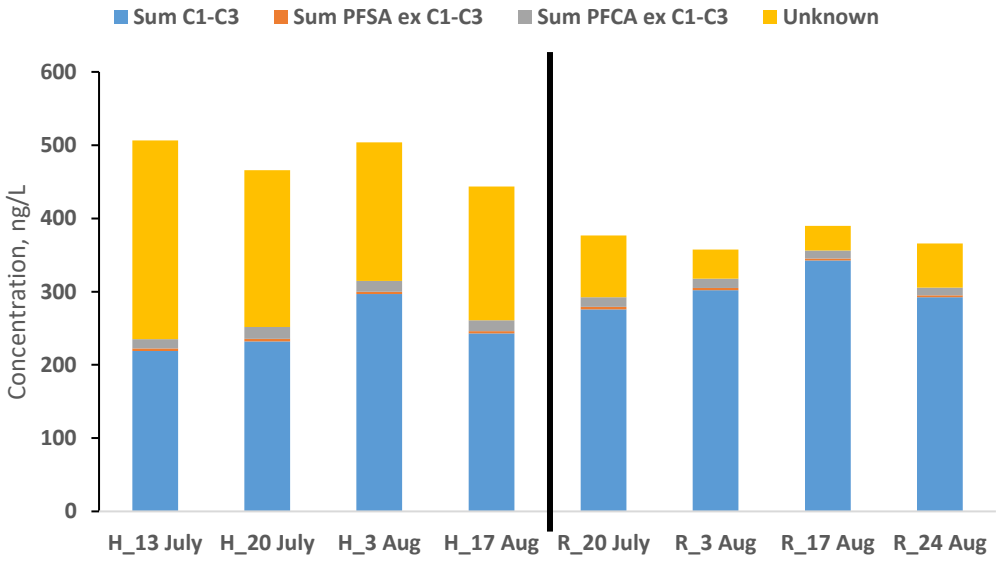

Figure 4. Occurrence of extractable organofluorine (EOF) in irrigation water from two different sampling locations in July and August, 2021. The fluorine represented by C1-C3 perfluoroalkyl acids, perfluoroalkyl sulfonic acids (PFSA) and perfluoroalkyl carboxylic acids (PFCA) is given. H - Hemse, R - Roma

Further analysis of the irrigation water to elucidate the unknown fraction of EOF was done by TOPA. The oxidation products can reveal the presence of precursors not included in the target analysis. In the current analysis, only oxidation products with carbon chain length four or higher could be determined. Only four samples showed an increase of at least 25% after oxidation, given in Figure 5 is the comparison of before and after oxidation for PFHxA and PFOA, highlighting the four samples with a >25% increase. The identity of the unknown fraction of EOF could not be determined by TOPA, which might suggest that it is either non-PFAS substances or PFAS that does not oxidize to C4-C14 acids.

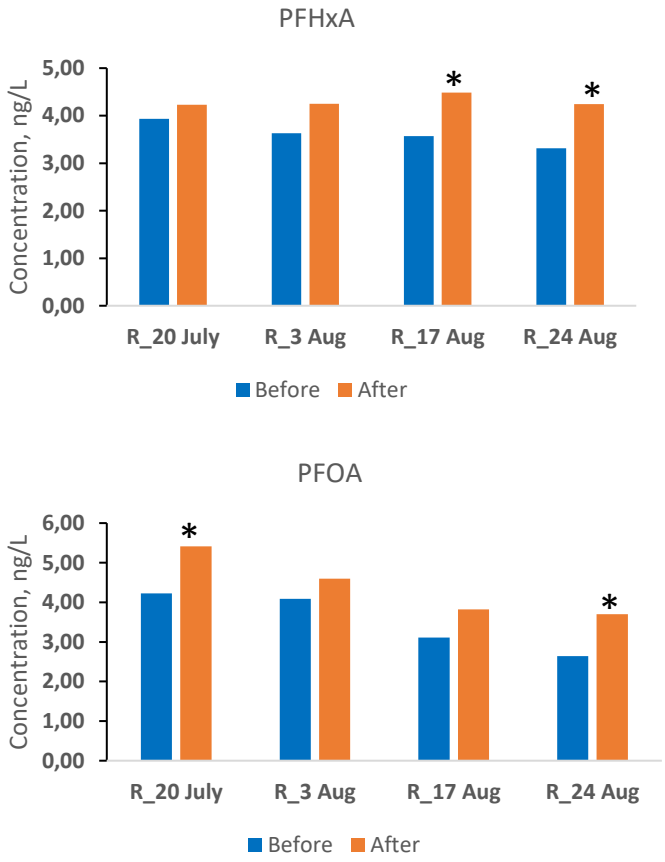

Figure 5. Concentrations (ng/L) of PFHxA and PFOA in irrigation water before and after oxidation using the TOPA method. H - Hemse, R – Roma. Indicated by * is an increase after oxidation that exceeds 25%.

3.2 Soil, earthworms and potatoes

3.2.1 OMPs

Soil samples and earthworms from the irrigated field, and samples of potatoes grown in soil treated with the treated effluent water were evaluated. It is important to note that none of the OMPs in this study was detected in any of the soil samples. It could be explained by the fact that OMPs could degrade in the irrigated soil during the growing season [26].

Out of 77 analyzed OMPs, only five OMPs (sotalol, oxazepam, metformin, ethylparaben and venlafaxine) were found in earthworms, Figure 6, Table A4.1 in appendix. Metformin, oxazepam and sotalol were found in worms from the reference site in Hemse, with a sum concentration of 180 ng/g d.w. The analysis of earthworm samples showed big variation in concentration levels from sampling locations Hemse and Roma. This can be due to high concentration levels of OMPs in irrigated water used from location Hemse.

Only two compounds (metformin, 6.6 ng/g d.w. and BAM 340 ng/g d.w.) were found in potatoes collected from location Roma. This fact confirmed the translocation and accumulation of OMPs to edible plants; therefore, the human and animal exposure to these compounds strongly depends on compounds physicochemical properties (i.e. hydrophobicity and electrical charge), as well as on the crop type and plant's physiology, as previously reported [2, 26].

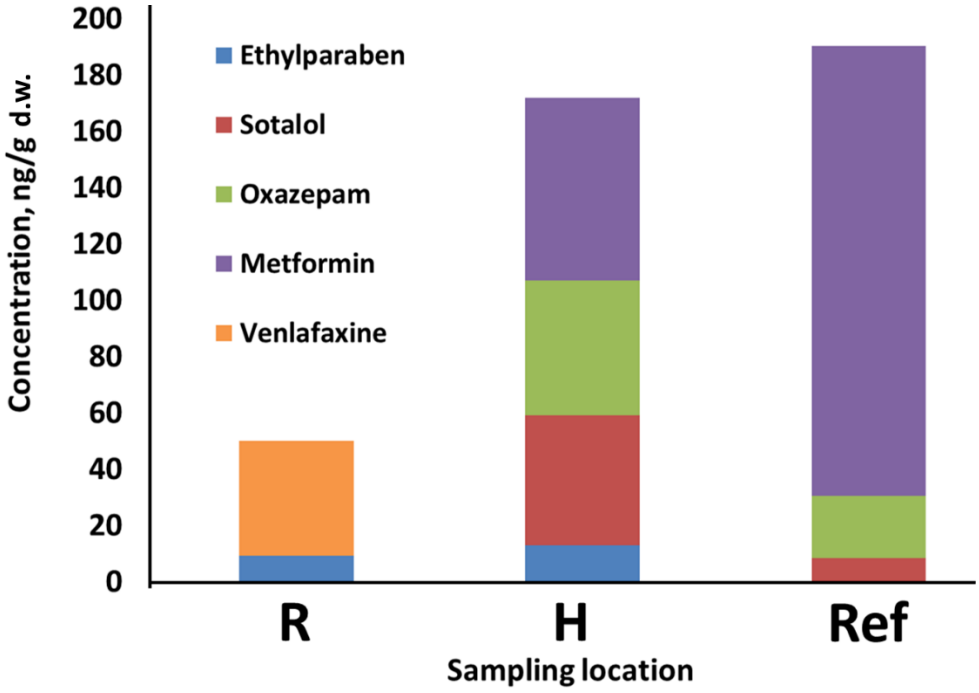

Figure 6. Occurrence of detected OMPs in earthworms, from three different sampling locations, September 2021. R= Roma, H= Hemse, Ref= reference site in Hemse, not affected by wastewater irrigation

It is important to highlight the presence of metformin in potatoes and earthworm samples, demonstrating a translocation and accumulation in the plant and biota. Metformin was also one of the main contaminant in irrigated water with average concentration of 207 ng/L in sampling location Hemse and 49 ng/L in sampling location Roma, which can partially explain the high concentrations reached in the worm location Hemse respectively.

Uptake and accumulation of contaminants into earthworms not only poses a risk to the earthworm directly but bioaccumulation and contaminant transfer through the food chain to top predators such as birds yield the potential for secondary poisoning. Previous research [27] has demonstrated that earthworms can biomagnify inorganic and organic soil contaminants. Given the paucity of the data and the potential for pharmaceuticals to end up in the soil, further research is therefore required to fully characterize the potential for pharmaceutical uptake into terrestrial invertebrates.

3.2.2 PFAS

Two PFAS were detected in soil. PFOS (only the linear isomer) was found close to detection limit at both Hemse and Roma (0.14-0.17 ng/g d.w.) but was below detection limit at the reference site. PFBA was found at low levels close to detection limit in Hemse (0.22 ng/g d.w.) and the reference site in Hemse (0.27 ng/g d.w.). The EOF analysis of soil did not result in any further information due to an elevated detection limit (277 ng/g d.w.). Concentrations and detection limits can be found in Table A3 in appendix.

The potato sample from Roma did not contain any PFAS or EOF above detection limit (Table A5 in appendix). It should be noted that the EOF detection limit was elevated (328 ng/g d.w.) for potatoes as well as for soil and that there is a need for method improvements.

Ten PFAS were detected in earthworms from Roma with the sum concentration 35 ng/g d.w. and five were detected in Hemse, with sum concentration 19 ng/g d.w. Seven PFAS was found in worms from the reference site in Hemse, with a sum concentration of 69 ng/g d.w. Concentrations are provided in Table A4.2 in appendix and the PFAS homologue pattern in earthworms is shown in Figure 7. Most of the PFAS found in earthworms from Roma and Hemse were long chain acids, including PFOS, that are known to bioaccumulate. Exceptions are PFBA that was found at both locations, and PFPrA that was found in worms from the reference site. The bioaccumulation of perfluoroalkyl acids in earthworms from contaminated soil has been shown to generally increase with increasing chain length for perfluoroalkyl acids (PFCA) while limited influence or decrease with perfluorinated chain length was observed for sulfonic acids (PFSA) [28]. Roma shows the highest levels of the two sites, but the levels in earthworms from the reference site in Hemse exceeds both the irrigated sites. Although the homologue pattern differs between all three locations, the reference site stands out by substantial contributions of PFPrA and PFHxS, with 32% and 10% of the sum of PFAS.

The compound present at highest concentration in irrigation water, TFA, was not detected in soil, earthworms or potatoes.

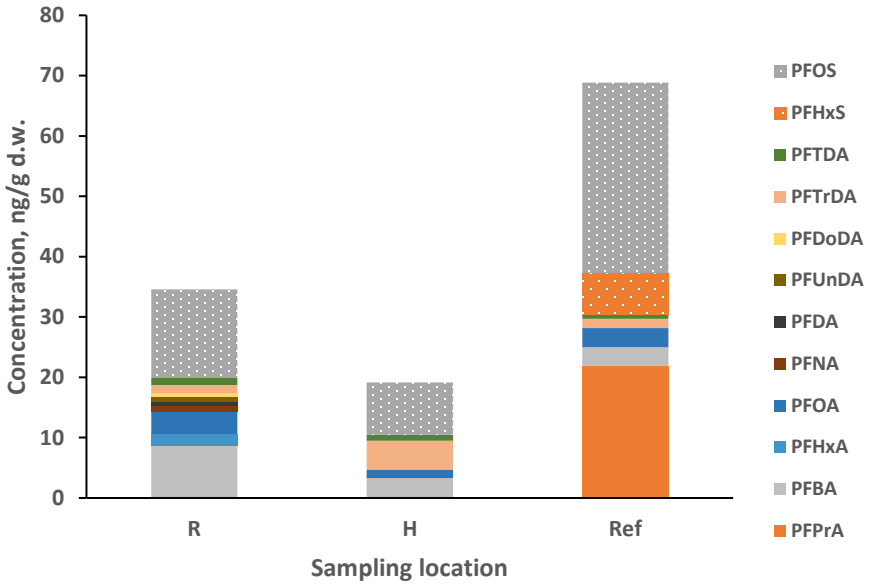

Figure 7. Occurrence of detected PFAS in earthworms, collected from three different sampling locations, September 2021. R= Roma, H= Hemse, Ref= reference site in Hemse, not affected by wastewater irrigation

Possible link between PFAS in irrigation water and PFAS in earthworms cannot be distinguished by comparing the homologue patterns (Figure 8). It should be noted that field bioaccumulation potential will affect the PFAS profile in worms, and that it is influenced by soil characteristics as well as occurrence of precursors that can transform to stable end-products. A direct comparison between irrigation water and biota can therefore not be made. While PFAS with high water solubility is expected to be present in highest concentration in effluent water (left part of the x-axis of Figure 8), accumulation in biota is favored by hydrophobic interactions that is determined by several factors, increased carbon chain length being one factor (right side of Figure 8). The reference site is clearly affected by PFAS contamination, resulting in higher concentrations in earthworms compared to the irrigated sites. The reason for this is unknown.

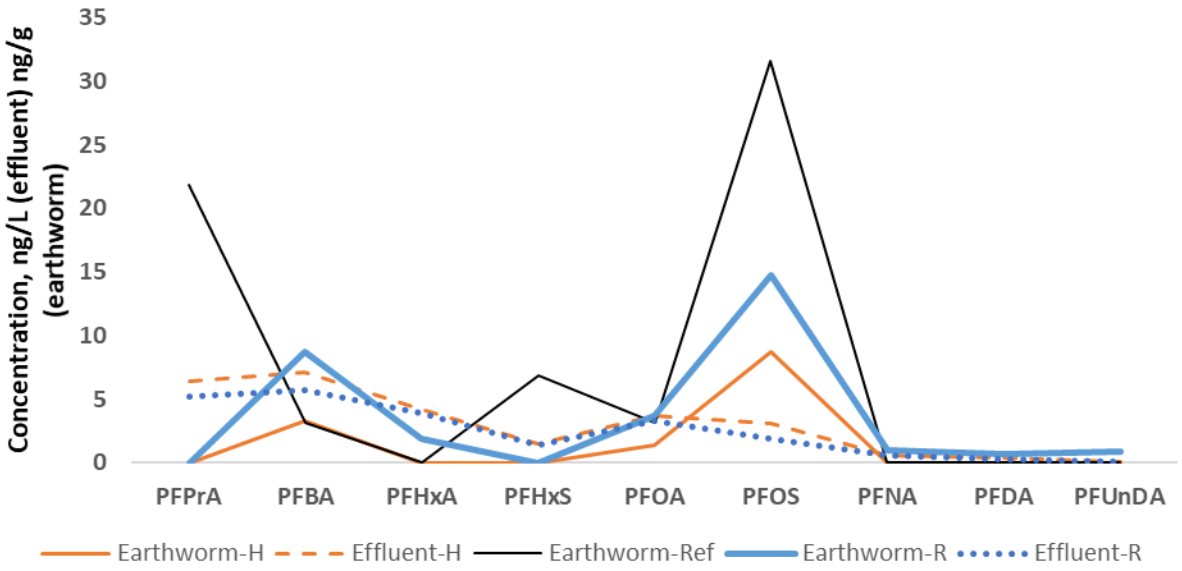

Figure 8. PFAS detected in both earthworms (ng/g d.w.) and effluent (irrigation water, ng/L) from Hemse (H), reference site in Hemse (Ref) and Roma (R). Substances are sorted with increasing perfluorinated chain length from left to right.

4. Conclusions.

A total of 54 contaminants were detected in at least one sample, in mean concentrations ranging from ng/L to µg/L in irrigated water samples and from ng/g to µg/g in biota samples.

The current study did not show a risk of OMPs for uptake in edible plants (potatoes) from soil irrigated with treated wastewater effluent. Residue degradation, formation of soil-bound residues and loss by leaching are factors that could limit the availability of OMPs residues for subsequent uptake from the soil into crops. There is a need for evaluation of crop OMPs uptake from soil irrigated with treated effluent by composting. In summary, the present study revealed no significant uptake of OMPs that vary widely in their chemical properties into potatoes.

The effluent water used for irrigation contained several PFAS in concentrations that have been typically found in Swedish municipality effluent water. The target compounds found in irrigation water could not be detected at substantial levels in neither soil nor potatoes. The reason for this could be that irrigation water contains mostly polar PFAS that is leachable from soil. The levels in soil could also be lower than the current detection limits, considering that the detection limits in water are approximately a factor of 1000 lower compared to soil. Uptake of PFAS from irrigation water could still occur, and other crops besides potatoes, e.g. plants with the edible part above ground, might be able to accumulate more polar chemicals. A number of PFAS were detected in earthworms but with no clear link to the irrigation water. The PFAS profile in effluent water was relatively similar between the two locations; however, the profiles in earthworms from the included sites differed. The impact of PFAS in irrigation water to farmland was further challenging to interpret since the reference location also was influenced by PFAS contamination with higher levels in earthworms compared to the irrigated lands.

Even though there are many factors that influence the uptake of OMPs in food crops under real field conditions (irrigation water quality, irrigation system, proximity to traffic roads, industrial runoff and fertilizer applications among others), results can be extrapolated to other regions, considered a very vulnerable scenario in climate change forecasts, with significant and increasing risks for water, ecosystems, food, health and security interconnected domains.

Acknowledgements
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Appendix

Table A1.1. List of included OMPs in the study

Compound	Category	Type
Acetaminophen	Pharmaceutical	Analgesics (painkiller)
Albuterol (Salbutamol)	Pharmaceutical	Beta blocker
Amitriptyline	Pharmaceutical	Antidepressant
Amoxicillin	Pharmaceutical	Antibiotic
Atenolol	Pharmaceutical	Beta blocker
Atovastatin (Lipitor)	Pharmaceutical	Antilipidemic Agents
Azithromycin	Pharmaceutical	Antibiotic
BAM (Dichlorobenzamide)	Pesticide	Metabolite of dichlobenil
Bezafibrate	Pharmaceutical	Antilipemic drug
Bicalutamide	Pharmaceutical	Antineoplastic agent
Bisoprolol	Pharmaceutical	Antihypertensive
Caffeine	Stimulant	
Carazolol	Pharmaceutical	Beta blocker
Carbamazepine	Pharmaceutical	Antiepileptic
Cetirizine	Pharmaceutical	Antihistamine
Chloramphenicol	Pharmaceutical	Antibiotic
Chlorzoxazone	Pharmaceutical	Muscle relaxant?
Ciprofloxacin	Pharmaceutical	Antibiotic (quinolone)
Citalopram	Pharmaceutical	Antidepressant
Clarithromycin	Pharmaceutical	Antibiotic
Climbazole	Pharmaceutical	Antifungal
Clindamycin	Pharmaceutical	Antibiotic
Clozapine	Pharmaceutical	Antipsychotic
Codeine	Pharmaceutical	Opiates, opioids and metabolites
Daidzein	Isoflavone	
DEET	Pesticide	Insect repellent
Desvenlafaxine	Pharmaceutical	Antidepressant
Diazepam	Pharmaceutical	Sedative
Diclofenac	Pharmaceutical	NSAID (nonsteroidal anti-inflammatory drug)
Diltiazem	Pharmaceutical	Antihypertensive
Erythromycin	Pharmaceutical	Antibiotic
Ethylparaben	Paraben	Antifungal preservative
Fexofenadine	Pharmaceutical	Antihistamine
Fluconazole	Pharmaceutical	Antifungal
Fluoxetine	Pharmaceutical	Antidepressant
Furosemide	Pharmaceutical	Diuretics
Gemfibrozil	Pharmaceutical	Antilipidemic Agents
Hydrochlorothiazide (HCTZ)	Pharmaceutical	Diuretics

Ifosfamide	Pharmaceutical	Anticancer
Irbesartan	Pharmaceutical	Antihypertensive
Lamotrigine	Pharmaceutical	Antiepileptic
Lidocaine	Pharmaceutical	Anesthetic
Loperamide	Pharmaceutical	Antidiarrhoeal
Losartan	Pharmaceutical	Antihypertensive
Memantine	Pharmaceutical	Alzheimer
Metformin	Pharmaceutical	Biguanide hypoglycemic agent (non-insulin-dependent diabetes mellitus)
Methylparaben	Paraben	Antifungal preservative
Metoprolol	Pharmaceutical	Beta blocker
Metronidazole	Pharmaceutical	Antibiotic
Mirtazapine	Pharmaceutical	Antidepressant
Nicotine	Stimulant	
Norsertraline	Pharmaceutical	Antidepressant
Omeprazole	Pharmaceutical	Antisecretory Agent
Oxazepam	Pharmaceutical	Sedative
Oxycodone	Pharmaceutical	Opiates, opioids and metabolites
Panthenol	Pharmaceutical	Moisturizer
Paroxetine	Pharmaceutical	Antidepressant
Primidone	Pharmaceutical	Antiepileptic
Propranolol	Pharmaceutical	Beta blocker
Propylparaben	Paraben	Antifungal preservative
Pyrimethamine	Pharmaceutical	
Ramipril	Pharmaceutical	Antihypertensive
Ranitidine	Pharmaceutical	Antisecretory Agent
Roxithromycin	Pharmaceutical	Antibiotic
Salicylic acid	Pharmaceutical	NSAID (nonsteroidal anti-inflammatory drug)
Sertraline	Pharmaceutical	Antidepressant
Simvastatin	Pharmaceutical	Statins (HMG CoA reductase inhibitors)
Sotalol	Pharmaceutical	Beta blocker
Sulfamethoxazole	Pharmaceutical	Antibiotic
Tamoxifen	Pharmaceutical	Anticancer
Terbutaline	Pharmaceutical	Beta adrenergic receptor agonists
Thiabendazole	Pharmaceutical	Antihelminthic
Tolytriazole	Pharmaceutical	
Tramadol	Pharmaceutical	Analgesics (painkiller)
Trimethoprim	Pharmaceutical	Antibiotic
Valsartan	Pharmaceutical	Antihypertensive
Venlafaxine	Pharmaceutical	Antidepressant

Table A1.2. List of included PFAS in the study

Perfluoroalkyl carboxylic acids (PFCA)	Trifluoroacetate (TFA)
	Perfluoropropanoic acid (PFPrA)
	Perfluorobutanoic acid (PFBA)
	Perfluoropentanoic acid (PFPeA)
	Perfluorohexanoic acid (PFHxA)
	Perfluoroheptanoic acid (PFHpA)
	Perfluorooctanoic acid (PFOA)
	Perfluorononanoic acid (PFNA)
	Perfluorodecanoic acid (PFDA)
	Perfluoroundecanoic acid (PFUnA)
	Perfluorododecanoic acid (PFDoDA)
	Perfluorotridecanoic acid (PFTrDA)
	Perfluorotetradecanoic acid (PFTeDA)
Perfluoroalkyl sulfonic acid (PFSA)	Trifluoromethane sulfonic acid (TFMS)
	Perfluoroethane sulfonic acid (PFEtS)
	Perfluoropropate sulfonic acid (PFPrS)
	Perfluorobutane sulfonic acid (PFBS)
	Perfluorohexane sulfonic acid (PFHxS)
	Linear Perfluorooctane sulfonic acid (lin-PFOS)
	Branched Perfluorooctane sulfonic acid (br-PFOS)
	Perfluorodecane sulfonic acid (PFDS)
Fluoroalkylsulfonamide	Perfluorooctane sulfonamide (FOSA)
Fluorotelomer sulfonic acid	4:2 Fluorotelomer sulfonic acid (4:2 FTSA)
	6:2 Fluorotelomer sulfonic acid (6:2 FTSA)
	8:2 Fluorotelomer sulfonic acid (8:2 FTSA)

Table A2.1 Concentrations (ng/L) of positive OMPs in effluent collected for irrigation, 2021

Site	Hemse 13/7	Hemse 20/7	Hemse 3/8	Hemse 17/8	Roma 20/7	Roma 3/8	Roma 17/8	Roma 24/8
Atenolol	2.3	< 1.1	< 1.1	< 1.1	< 1.1	< 1.1	< 1.1	< 1.1
Sotalol	5	7.7	3	< 1.1	14	1.9	3.3	6.7
Nicotine	11	180	37	5.6	10	18	11	10
Metoprolol	31	12	16	3.4	5.4	7.7	10	3.8
Carbamazepine	150	140	160	28	27	39	150	31
Cetirizine	39	43	42	7.2	4.4	10	38	8.1
Citalopram	3.7	3.8	4.3	2.3	< 1.1	< 1.1	3.3	< 1.1
Oxazepam	44	47	34	3.9	2.1	5.1	23	7.2
Lamotrigine	460	380	440	110	140	190	310	150
Metformin	410	260	150	9.1	8.5	33	140	14
DEET	51	70	59	30	34	46	55	29
Bezafibrate	< 1.1	2.2	< 1.1	< 1.1	< 1.1	< 1.1	< 1.1	< 1.1
Bicalutamide	160	200	160	4.1	3.7	5.8	180	4.7
Bisoprolol	4.3	2	< 1.1	< 1.1	< 1.1	< 1.1	< 1.1	< 1.1
Clarithromycin	5.8	16	5.8	< 1.1	< 1.1	< 1.1	9.5	< 1.1
Fexofenadine	18	21	15	13	7.5	17	14	14
Memantine	4.1	2	3.2	2.6	3.5	6.5	< 1.1	2.8
Caffeine	92	130	120	81	110	190	110	100
Tramadol	27	24	26	< 3.8	< 3.8	4.8	14	< 3.8
Valsartan	< 6.1	< 6.1	7.5	< 6.1	< 6.1	< 6.1	< 6.1	< 6.1
Fluconazole	67	86	65	14	15	24	71	13
Diclofenac	< 1.1	64	14	< 1.1	< 1.1	< 1.1	< 1.1	< 1.1
Ethylparaben	8	4.5	8.3	< 1.9	27	< 1.9	6.6	9.4
Methylparaben	5.6	7.2	8.7	6.6	8.9	17	3.2	6
Furosemide	< 1.1	13	10	2.9	14	< 1.1	< 1.1	< 1.1
Losartan	77	92	33	6.6	5.2	6.6	21	10
Metronidazole	< 1.1	9.7	< 1.1	< 1.1	< 1.1	< 1.1	< 1.1	< 1.1
Sulfamethoxazole	11	30	7.8	< 1.1	< 1.1	< 1.1	12	< 1.1
Trimethoprim	13	13	12	< 1.1	< 1.1	< 1.1	8.2	< 1.1
Amitriptyline	8.8	7.4	6.7	7.6	8	11	< 1.1	6.8
Venlafaxine	26	21	23	< 1.1	3.4	6	17	4.4
Desvenlafaxine	< 1.1	< 1.1	2.7	< 1.1	< 1.1	< 1.1	< 1.1	< 1.1
Tolytriazole	36	37	37	11	12	21	32	15
Panthenol	< 1.1	< 1.1	< 1.1	< 1.1	< 1.1	3	< 1.1	< 1.1
Salicylic acid	4	7.7	< 1.1	5.1	7	4.8	4	< 1.1

Table A2.2 Concentrations (ng/L) of PFAS and EOF in effluent collected for irrigation, 2021

	Roma 20/7	Roma 8/7	Roma 17/8	Roma 24/8	Hemse 13/7	Hemse 20/7	Hemse 3/8	Hemse 17/8
EOF	377	358	390	366	507	466	504	444
TFA	1099	1390	1229	1289	858	846	894	1011
PFPrA	5.3	5.0	5.2	5.3	4.9	5.5	7.6	7.6
PFBA	6.1	6.4	5.3	5.1	6.4	7.3	7.2	7.5
PFPeA	3.2	3.2	2.9	3.0	2.8	3.2	3.4	3.5
PFHxA	4.1	4.0	3.7	3.9	3.7	4.1	4.4	4.5
PFHpA	2.1	2.0	1.9	2.0	2.4	2.6	2.6	2.7
PFOA	3.9	4.0	2.9	2.6	3.3	4.2	3.8	3.5
PFNA	0.80	0.61	0.34	0.38	0.51	0.65	0.55	0.52
PFDA	0.37	0.26	0.12	0.20	0.37	0.46	0.36	0.31
PFUnDA	0.06	0.05	<0.02	0.04	0.04	0.06	0.04	0.08
PFDoDA	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	0.1
PFTrDA	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06
PFTDA	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
TFMS	3.3	4.5	3.9	3.4	4.1	4.4	4.7	5.1
PFEtS	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
PFPrS	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
PFBS	0.77	0.84	0.86	0.87	0.75	0.83	0.73	0.85
PFPeS	0.28	0.23	0.18	0.22	0.21	0.25	0.22	0.20
PFHxS	1.4	1.4	1.3	1.3	1.5	1.6	1.5	1.5
PFHpS	<0.03	<0.03	<0.03	<0.03	0.1	<0.03	<0.03	<0.03
L-PFOS	1.6	1.2	0.6	1.0	1.8	2.2	1.9	1.7
Br-PFOS	0.88	0.91	0.71	0.80	1.1	1.2	1.2	1.4
PFNS	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03
PFDS	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PFDoDS	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
FOSA	0.07	<0.06	<0.06	<0.06	0.08	<0.06	<0.06	<0.06
4:2 FTSA	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03
6:2 FTSA	<0.06	0.06	<0.06	<0.06	0.10	0.08	0.06	0.12
8:2 FTSA	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06

Table A3. Concentrations (ng/g d.w.) of PFAS and EOF in soil (0-17 cm, n=3 replicates), collected in September 2021

Site	Roma	Roma	Roma	Hemse	Hemse	Hemse	Ref. site	Ref. site	Ref. site
Water content (%)	21			14			14		
EOF	<277	<277	<277	<277	<277	<277	<277	<277	<277
TFA	<1	<1	<1	<1	<1	<1	<1	<1	<1
PFPrA	<1	<1	<1	<1	<1	<1	<1	<1	<1
PFBA	<0.10	0.11	<0.10	0.22	0.22	0.22	0.27	0.26	0.27
PFPeA	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
PFHxA	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16
PFHpA	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PFOA	<0.22	<0.22	<0.22	<0.22	<0.22	<0.22	<0.22	<0.22	<0.22
PFNA	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
PFDA	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PFUnDA	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
PFDoDA	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
PFTrDA	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PFTDA	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
TFMS	<1	<1	<1	<1	<1	<1	<1	<1	<1
PFEtS	<1	<1	<1	<1	<1	<1	<1	<1	<1
PFPrS	<1	<1	<1	<1	<1	<1	<1	<1	<1
PFBS	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PFPeS	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
PFHxS	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
PFHpS	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
L-PFOS	0.15	0.17	0.16	0.14	0.16	0.14	<0.13	<0.13	<0.13
Br-PFOS	<0.19	<0.19	<0.19	<0.19	<0.19	<0.19	<0.19	<0.19	<0.19
PFDS	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
FOSA	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
4:2 FTSA	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
6:2 FTSA	<3.4	<3.4	<3.4	<3.4	<3.4	<3.4	<3.4	<3.4	<3.4
8:2 FTSA	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10

Table A4.1 Concentrations (ng/g d.w.) of positive OMPs in earthworms (n=3 replicates), collected in September 2021

Site	Roma	Hemse	Ref. Site
Ethylparaben	9.2	13	< 8.7
Sotalol	< 1.8	46	8.4
Oxazepam	< 6.8	48	22
Metformin	< 58	65	160
BAM (Dichlorobenzamide)	<53	<53	<53
Venlafaxine	41	< 17	< 17

Table A4.2 Concentrations (ng/g d.w.) of PFAS and EOF in earthworms (n=3 replicates), collected in September 2021

Site	Roma	Roma	Roma	Hemse	Hemse	Hemse	Ref. site	Ref. site	Ref. site
Water content (%)	91			89			89		
EOF	<1500	<1500	<1500	<1500	<1500	<1500	<1500	<1500	<1500
TFA	<1	<1	<1	<1	<1	<1	<1	<1	<1
PFPrA	<1	<1	<1	<1	<1	<1	21.9	20.8	22.9
PFBA	8.30	9.27	8.64	2.87	3.31	3.64	3.53	2.83	3.08
PFPeA	<0.13	<0.13	<0.13	<0.13	<0.13	<0.13	<0.13	<0.13	<0.13
PFHxA	1.76	1.89	2.00	<0.71	<0.71	<0.71	<0.71	<0.71	<0.71
PFHpA	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.
PFOA	3.5	3.8	3.9	1.3	1.5	1.5	3.2	3.0	3.3
PFNA	0.91	0.88	1.0	<0.13	<0.13	<0.13	<0.13	<0.13	<0.13
PFDA	0.65	0.74	0.71	<0.34	<0.34	<0.34	<0.34	<0.34	<0.34
PFUnDA	0.70	0.92	0.87	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
PFDoDA	0.56	0.61	0.63	<0.13	<0.13	<0.13	<0.13	<0.13	<0.13
PFTrDA	1.2	1.4*	1.5	4.4	4.5	5.5	1.0*	2.1*	1.6*
PFTDA	1.2	0.94*	1.3	1.1	0.94	0.92	0.78	0.50	0.80
TFMS	<1	<1	<1	<1	<1	<1	<1	<1	<1
PFEtS	<1	<1	<1	<1	<1	<1	<1	<1	<1
PFPrS	<1	<1	<1	<1	<1	<1	<1	<1	<1
PFBS	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00
PFPeS	<0.11	<0.11	<0.11	<0.11	<0.11	<0.11	<0.11	<0.11	<0.11
PFHxS	<4.0	<4.0	<4.0	<4.0	<4.0	<4.0	6.8	6.5	7.3
PFHpS	<0.12	<0.12	<0.12	<0.12	<0.12	<0.12	<0.12	<0.12	<0.12
L-PFOS	14.5	14.8	14.9	8.8	8.3	8.9	26.3	27.7	26.7
Br-PFOS	<0.17	<0.17	<0.17	<0.17	<0.17	<0.17	4.7	4.8	4.5
PFDS	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

FOSA	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25
4:2 FTSA	<0.12	<0.12	<0.12	<0.12	<0.12	<0.12	<0.12	<0.12	<0.12
6:2 FTSA	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3
8:2 FTSA	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24

* Interferences (ion ratio not approved) but deemed accurate

n.q. Interferences (ion ratio not approved), could not be quantified

Table A5. Concentrations (ng/g d.w.) of PFAS in potatoes (n=3 replicates), collected in September 2021

Site	Roma	Roma	Roma
Water content (%)	79		
EOF	<328	<328	<328
TFA	<1	<1	<1
PFPrA	<1	<1	<1
PFBA	<0.10	<0.10	<0.10
PFPeA	<0.05	<0.05	<0.05
PFHxA	<0.16	<0.16	<0.16
PFHpA	<0.10	<0.10	<0.10
PFOA	<0.22	<0.22	<0.22
PFNA	<0.05	<0.05	<0.05
PFDA	<0.10	<0.10	<0.10
PFUnDA	<0.05	<0.05	<0.05
PFDoDA	<0.05	<0.05	<0.05
PFTrDA	<0.10	<0.10	<0.10
PFTDA	<0.05	<0.05	<0.05
TFMS	<1	<1	<1
PFEtS	<1	<1	<1
PFPrS	<1	<1	<1
PFBS	<0.10	<0.10	<0.10
PFPeS	<0.05	<0.05	<0.05
PFHxS	<0.05	<0.05	<0.05
PFHpS	<0.12	<0.12	<0.12
L-PFOS	<0.13	<0.13	<0.13
Br-PFOS	<0.19	<0.19	<0.19
PFDS	<0.20	<0.20	<0.20
FOSA	<0.10	<0.10	<0.10
4:2 FTSA	<0.05	<0.05	<0.05
6:2 FTSA	<3.4	<3.4	<3.4
8:2 FTSA	<0.10	<0.10	<0.10